PLANT PHYSIOLOGY

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VOLUME II

1927

WITH FIVE PLATES AND EIGHTY-SIX FIGURES

SCIENCE PRESS PRINTING CO.
LANCASTER, PA.

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ERRATA, VOLUME II

Page 132, line 5, for investigation read investigations.
Page 164, line 14, for Schaffer read Shaffer.
Page 179, line 1, for is read in.
Page 205, line 8, for (10) read (12).
Page 206, line 14, for (11) read (13).
Page 205, line 35, for (6) read (6, 7).
Page 207, line 18, for (9) read (10, 11).
Page 208, line 14, for (8) read (9).
Page 209, line 19, for (7) read (8).
Page 221, line 4, for wil read will.
Page 224, line 7, for soils read sols.

Vol. II No. 1

PLANT PHYSIOLOGY

JANUARY, 1927

NEGATIVE RESULTS ON PHYSIOLOGICAL BALANCE IN SOIL CULTURES*

R. P. HIBBARD

(WITH ELEVEN FIGURES)

Introduction

The use in the field of the triangular system of determining salt ratios was first attempted by the writer in the growing season of 1918. In the fall of that year, SCHREINER and SKINNER (**10**) reported on some of their field work. It has been shown previously by the author (**3**) that there is some evidence of a physiological balance in the soil solution extracted from the soil and set up in culture jars in the greenhouse. The study reported here was expected to show whether or not a physiological balance exists in the soil solution *in situ*. It is perfectly evident that such a study as this is considerably more complicated than one in water cultures. The medium of growth, a fertile soil, is indeed very complex, physically, chemically, and biologically. It must also be considered as in a dynamic state, in a condition of change. In spite of the complexities which make correct interpretations difficult, and because there are still possibilities for improvement in the generally accepted plan of applying soil fertilizers, SCHREINER and SKINNER (**10**), LIPMAN and LINHART (**7**), efforts should be made and are being made to apply the triangular system to field studies. It is quite evident that for these reasons, if not for the fact that our annual expenditure for fertilizer practice is well over a hundred million dollars a year, any and every fertilizer practice should be actively investigated.

Such studies involve investigations of the salt requirements of agricultural plants, a study of the mechanism of absorption, and of the uses, functions and modifications of the various ions when finally within the plant.

* Published with the permission of the Director of the Experiment Station.

Greenhouse methods

The method of conducting this experiment is based on previous but unpublished studies of wheat, corn, and tomatoes grown in different media, soil, quartz sand, and distilled water (**4**).

The culture pots were 23 cm. high and 20.5 cm. in diameter, sufficiently large to hold enough soil (6 kilograms) to allow for the growth of wheat to maturity. In using pots of this size it is necessary to construct special watering devices, incorrectly designated by some authors as potometers. The apparatus has been frequently used in this laboratory for many years and is described by YUNCKER (**16**).

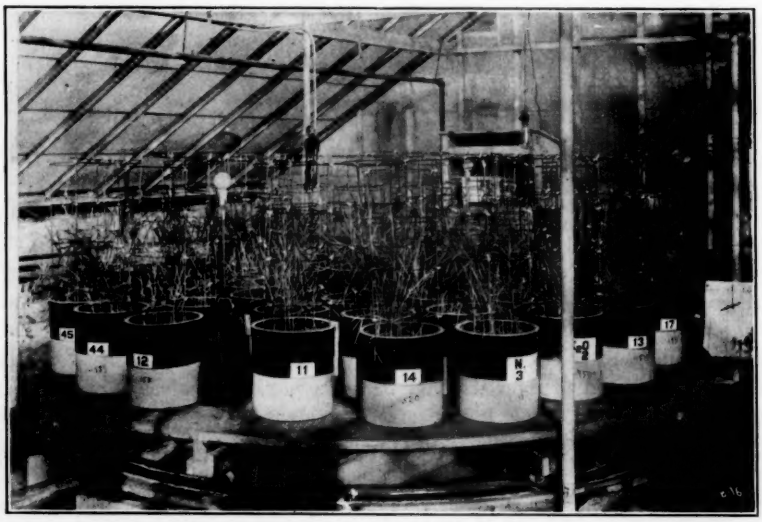

FIG. 1. Plant cultures in early stages of growth on rotating table. Temperature cage, evaporimeters, and humidity apparatus are shown.

For aerating the culture, a piece of glass tubing was run into the soil down to the gravel layer at the bottom of the crock. The old air was forced out and new introduced each time water was added through the watering device. Since the surface was not sealed with wax, evaporation from the soil was lessened by a top layer of fine gravelly loam. As a record of water lost from the plants was desired, the small amount lost through the soil had to be determined by comparing the amounts lost from three or four similarly prepared pots not planted to a crop.

The culture pots thus constructed were entirely satisfactory, and the plants grown in them were very vigorous and looked very healthy. They

compared very favorably with plants of the same variety in the field. Marquis wheat, the variety used here, grows to a height varying from 2.4 to 4 feet in the field. Those in the greenhouse varied from 3.2 to 3.8 feet. The heads were well formed, and varied in length from 2.7 inches to 3.8 inches, while in the field the variation is from 2.5 to 4 inches. In one respect, the plants in the field differed markedly from those in the greenhouse. In the field, Marquis averages 3 tillers to a plant, and all are likely to produce heads. But in the greenhouse, only 16 plants out of the 420 had tillers, and from these only one head was formed. The short duration of light, and its low intensity during the winter months when the experiment was conducted, probably account for the condition mentioned above (1). Two photographs of the plants were taken, one at an early stage of development, and the other at harvest time. These are shown in figures 1 and 2.

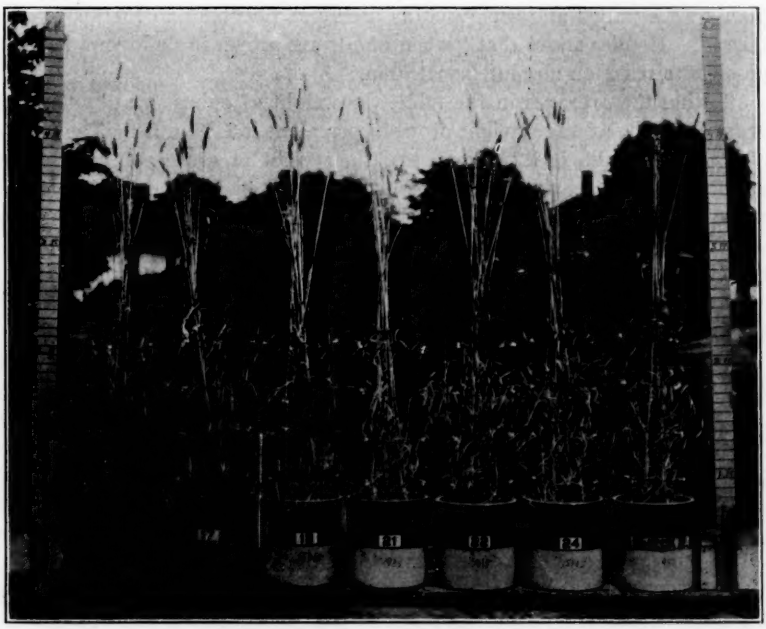

FIG. 2. Condition of plants at harvest time.

Comparison of an average field plant with an average plant grown in the greenhouse shows a close resemblance in all respects, except for the number of tillers. The field plants yield more grain because of the number of tillers. In table I are shown the field records of "Marquis wheat per

three square yards'' grown in Wisconsin and supplied to us by Professor ARNY.

TABLE I

MARQUIS WHEAT PER THREE SQUARE YARDS

PLOT NUMBER	NUMBER OF PLANTS	NUMBER OF CULMS	WEIGHT OF STRAW	WEIGHT OF GRAIN
			gm.	gm.
29	154	379	481.8	237.5
34	172	337	444.8	241.5
39	199	390	475.0	250.3
Average	175	369	467.2	243.1

In a letter dated November 15, 1920, Professor ARNY states that ''the plants were pulled and separated as nearly as possible and the above data taken.'' He also states that ''when plants are grown in drill rows it is not possible to make an absolute separation.''

Taking the averages in the table, the weight of culms per plant is 2.66 grams, and the average weight of grain per plant is 1.39 gram. The weight of straw per culm is 1.27 gram, and the weight of grain per culm, 0.656 gram. The average weight of culm calculated from our data is 1.44 gram, while for the 24 plants in the two check pots it is 1.26 gram. The average weight of grain per culm calculated from our data is 0.671 gram, while for the checks it is 0.598 gram. We may therefore safely conclude that the culture pots were suitably constructed for the growth of Marquis wheat in the greenhouse.

THE SOIL.—This was obtained from the field where the outdoor experiment was conducted. It was a composite sample of surface soil taken from a depth of not more than four inches from various parts of the field. In technical terms this soil is called a fine sandy loam of the Miami Series. At the time of the experiment, the moisture content of the soil was 1.11 per cent., the water-holding capacity 38.29 per cent., and the organic matter 4.95 per cent. These data enabled me to determine the amount of water to add to the pots to allow for excellent growth of the plants.

FERTILIZERS.—The three salts commonly used in the United States for fertilizer mixtures are $Ca(H_2PO_4)_2$, $NaNO_3$, and K_2SO_4. An analysis of each of the fertilizer salts was necessary in order to determine the exact amount of active ingredients present. Potassium sulphate contained 47.79 per cent. of K_2O, sodium nitrate 15.1 per cent. of nitrogen, and calcium acid phosphate 16.5 per cent. of available P_2O_5.

In the selection of fertilizer compounds, it is obvious that they should contain all of the essential elements, and that the compounds should be

compatible. In the sodium salt, we are introducing the Na ion which is not classed among those essential. The essential Mg ion is not present in any compound. It is assumed here that the Mg ion present in the soil is sufficiently abundant and need not be added as a commercial fertilizer. However, if water culture experiments were being planned, Mg would be necessary, and modification of the experiment would be advisable.

LIVINGSTON and TOTTINGHAM (**9**) have shown that there are differences in the growth of plants, depending on which basic and acidic radicals are united.

They have shown, in a preliminary test, that when KNO_3 is substituted for $Ca(NO_3)_2$, and when the H_2PO_4 ion is united with the Ca ion, better growth results. They suggested a thorough testing of the six possible combinations of the six essential elements of the ions Ca, Mg, NO_3, H_2PO_4, K, and SO_4.

FERTILIZER MIXTURES.—After studying the literature on fertilizer for wheat, it was decided to make the application at the rate of 100 pounds of active ingredients to the acre. Regardless of the filler in the fertilizer and regardless of the ratio of the various ingredients to each other, this means that the total weight of P_2O_5, K_2O and N in the mixture must always be 100. Calculation showed that the above application per acre would require, in the case of each pot of 2-gallon capacity, 320 milligrams of active ingredients. The actual amounts of the different ingredients for each pot or culture was determined in the following manner: The total weight (320 milligrams) was distributed among the three salts in such a manner as always to sum up to ten, since it had been arbitrarily determined that the proportions were to vary in ten per cent. increments. With three variables under consideration (N, P_2O_5, and K_2O) varying by increments of ten per cent., there are thirty-six possible combinations or ratios. For further details concerning the method of calculating the various ratios see TOTTINGHAM (**12**). The soil and fertilizers required for each pot were thoroughly mixed in the manner suggested by TRUOG (**14**). Besides the thirty-six cultures called for, twelve others were set up. Two were used as controls, four with an application of acid phosphate alone, three with sodium nitrate alone, and three with potassium sulphate alone. The quantities in grams in each of the ingredients, and for each fertilizer per pot are noted in table II.

SEEDS.—The seedlings for this experiment were raised from pure line Marquis wheat seeds supplied by W. E. TOTTINGHAM, of the Wisconsin Station, from the 1917 crop. The seeds were germinated on a paraffined mosquito netting over a dish of weak nutrient solution. When about six to nine centimeters tall those measuring 8 centimeters were transplanted into the pots, the seed part being buried to a depth of three-quarters of an inch. Each pot was planted to 12 seedlings. A recent study (**13**) has shown that

TABLE II

THE QUANTITIES OF P_2O_5, K_2O AND N PER POT

ACID PHOSPHATE 16.5 PER CENT. P_2O_5			SODIUM NITRATE 15.1 PER CENT. N		POTASSIUM SULPHATE 47.79 PER CENT. K_2O		TOTAL	
Pot number	P_2O_5 per pot	Fertilizer per pot	Nitrogen per pot	Fertilizer per pot	K_2O per pot	Fertilizer per pot	$P_2O_5 + N + K_2O$ per pot	$P_2O_5 + N + K_2O$ per acre
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	lbs.
1, 1, 8	0.032	0.194	0.032	0.212	0.256	0.536	0.32	100
1, 2, 7	0.032	0.194	0.064	0.424	0.224	0.469	0.32	100
1, 3, 6	0.032	0.194	0.096	0.636	0.192	0.402	0.32	100
1, 4, 5	0.032	0.194	0.128	0.848	0.160	0.335	0.32	100
1, 5, 4	0.032	0.194	0.160	1.060	0.128	0.268	0.32	100
1, 6, 3	0.032	0.194	0.192	1.272	0.096	0.201	0.32	100
1, 7, 2	0.032	0.194	0.224	1.484	0.064	0.134	0.32	100
1, 8, 1	0.032	0.194	0.256	1.696	0.032	0.067	0.32	100
2, 1, 7	0.064	0.388	0.032	0.212	0.224	0.469	0.32	100
2, 2, 6	0.064	0.388	0.064	0.424	0.192	0.402	0.32	100
2, 3, 5	0.064	0.388	0.096	0.636	0.160	0.335	0.32	100
2, 4, 4	0.064	0.388	0.128	0.848	0.128	0.268	0.32	100
2, 5, 3	0.064	0.388	0.160	1.060	0.096	0.201	0.32	100
2, 6, 2	0.064	0.388	0.192	1.272	0.064	0.134	0.32	100
2, 7, 1	0.064	0.388	0.224	1.484	0.032	0.067	0.32	100
3, 1, 6	0.096	0.582	0.032	0.212	0.192	0.402	0.32	100
3, 2, 5	0.096	0.582	0.064	0.424	0.160	0.335	0.32	100
3, 3, 4	0.096	0.582	0.096	0.636	0.128	0.268	0.32	100
3, 4, 3	0.096	0.582	0.128	0.848	0.096	0.201	0.32	100
3, 5, 2	0.096	0.582	0.160	1.060	0.064	0.134	0.32	100
3, 6, 1	0.096	0.582	0.192	1.272	0.032	0.067	0.32	100

TABLE II—(*Continued*)

THE QUANTITIES OF P_2O_5, K_2O AND N PER POT

Pot number	Acid phosphate 16.5 per cent. P_2O_5		Sodium nitrate 15.1 per cent. N		Potassium sulphate 47.79 per cent. K_2O		Total	
	P_2O_5 per pot	Fertilizer per pot	Nitrogen per pot	Fertilizer per pot	K_2O per pot	Fertilizer per pot	$P_2O_5 + N + K_2O$ per pot	$P_2O_5 + N + K_2O$ per acre
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	lbs.
4, 1, 5	0.128	0.776	0.032	0.212	0.160	0.335	0.32	100
4, 2, 4	0.128	0.776	0.064	0.424	0.128	0.268	0.32	100
4, 3, 3	0.128	0.776	0.096	0.636	0.096	0.201	0.32	100
4, 4, 2	0.128	0.776	0.128	0.848	0.064	0.134	0.32	100
4, 5, 1	0.128	0.776	0.160	1.060	0.032	0.067	0.32	100
5, 1, 4	0.160	0.970	0.032	0.212	0.128	0.268	0.32	100
5, 2, 3	0.160	0.970	0.064	0.424	0.096	0.201	0.32	100
5, 3, 2	0.160	0.970	0.096	0.636	0.064	0.134	0.32	100
5, 4, 1	0.160	0.970	0.128	0.848	0.032	0.067	0.32	100
6, 1, 3	0.192	1.164	0.032	0.212	0.096	0.201	0.32	100
6, 2, 2	0.192	1.164	0.064	0.424	0.064	0.134	0.32	100
6, 3, 1	0.192	1.164	0.096	0.636	0.032	0.067	0.32	100
7, 1, 2	0.224	1.358	0.032	0.212	0.064	0.134	0.32	100
7, 2, 1	0.224	1.358	0.064	0.424	0.032	0.067	0.32	100
8, 1, 1	0.256	1.552	0.032	0.212	0.032	0.067	0.32	100
Single fertilizers		1.552		1.696		0.536		
		0.776		0.848		0.268		
		0.388		0.424		0.134		
		0.194						
Check number 1, no fertilizer						Check number 2, no fertilizer		

uniform initial seed weight reduces variability, and that selection of young seedlings for uniformity of height exerts a similar influence, although probably less pronounced. Each culture was brought to a water content equal to 60 per cent. of its water-holding capacity. A layer of gravel covered the soil surface to reduce the surface loss of moisture. Each pot was then weighed and the weight recorded on the pot. This is necessary if one desires to know the water loss for definite periods of time.

ENVIRONMENTAL CONDITIONS.—The experiment was conducted in the greenhouse starting on December 16th and ending at harvest time, June 3rd. The cultures were placed on a large rotating table, 8 feet in diameter. There were too many pots to put in a single row so they were arranged in three rows as near the margin of the table as possible. The position of the cultures was changed from one row to another every three days. Some studies were made to determine the effect of the position of a culture on transpiration. It was found that cultures on the outer row transpired more water than those on the inner row and the middle row, while those on the inner row transpired more than those on the middle row. This was true for the period of this test or for about two weeks while the cultures were in the three rows. After this the cultures were set in two rows and the transpiration from them was not appreciably affected by whether the culture was in one or the other row. There were a few exceptions to this general rule and these were due to the fact that in one row there happened to be a larger proportion of cultures that were transpiring more heavily, and had so transpired from the beginning. In these tests on transpiration, it was further found that some cultures always transpired heavily no matter what row they were in. A few other cultures were low transpirers throughout the experiment.

At the start of the experiment the light intensity was probably not as conducive to good growth as might be desired, yet the plants did not appear to suffer. Shortly after the experiment was started, the days began to lengthen. When light became too intense during the latter part of the experimental period, cheese cloth was used to reduce the intensity, or the glass of the greenhouse was whitewashed. The temperature of the greenhouse for a period of nine weeks during the winter months was kept close to 70° F. by thermostatic regulation. Later in the season, with the steam off and the ventilators open, outdoor conditions were the rule, except at night when the ventilators were closed. During this period of five weeks, there were two low readings, and a few high ones (80° to 90° F.) at midday. During the last ten weeks, the temperature variations were more frequent and of longer duration. The plants, however, suffered at no time. The records show that 83 per cent. of the possible optimum range of tem-

perature for wheat was attained. This may be considered a very good percentage. A summary of the thermographic records is shown in table III.

TABLE III

SUMMARY OF THE THERMOGRAPHIC RECORDS FOR THE PERIOD OF THE EXPERIMENT

TWO-WEEK PERIODS	MAXIMUM	DURATION	MINIMUM	DURATION	DURATION ABOVE 70° F.	DURATION BELOW 55° F.	TOTAL DURATION 55°–70°	TOTAL DURATION 55°–70°
	°F	Hours	°F	Hours	Hours	Hours	Hours	Per cent.
Dec. 16–29 '18	87	0.5	60	0.5	15		321	95.5
Dec. 30–Jan. 12	87	0.5	56	0.5	18		318	94.6
Jan. 13–Jan. 26	92	0.5	62	0.5	16		302	95.2
Jan. 27–Feb. 9	78	0.5	49	0.5	12	1	323	96.0
Feb. 10–Feb. 23	82	1.0	54	0.5	17	1	318	94.6
Feb. 24–Mar. 9	75	0.5	42	0.5	20	41	275	81.9
Mar. 10–Mar. 23	88	1.0	49	0.5	37	6	293	87.2
Mar. 24–Apr. 6	97	0.5	38	0.5	76	49	211	62.8
Apr. 7–Apr. 20	89	0.5	48	1.0	27	35	274	81.5
Apr. 21–May 4	85	1.0	48	1.0	23	21	291	86.9
May 5–May 18	94	2.0	45	1.5	74	45	217	64.5
May 19–June 1	100*	1.5*	50	5.0	116	38	182	54.1
Total		10		12.5	451	197	3333	82.9

* Reached 100° at noon on six consecutive days for a period of 15 minutes each time.

The optimum range for wheat was taken as 55°–70° F., the range determined by HUTCHESON and QUANTZ (5) in their study on greenhouse temperatures as affecting the growth of small grains. The air temperature records were taken from a thermograph, shaded from the sun in a temperature cage.

That the air moisture condition in the greenhouse is an important factor in plant growth is well recognized, and a thorough wetting down of soil, benches, and walks daily is the usual practice. A too moist condition, however, is conducive to attacks of mildew, while a very dry greenhouse is unsuitable for plants, causing a drying of leaves, followed usually by the death of the plant, even though the roots are in moist soil or even in water cultures. The moisture condition of the air is usually given in terms of relative humidity, but unless the temperature is stated also the picture of the conditions is inadequate. The same percentages of relative humidity have very different meanings when the temperatures differ widely. Take, for example, an illustration from the present study. In the early part of the experiment, December 23rd, at 8 o'clock A. M., the relative humidity was 57 per cent. On June 7th at the same time of day, the relative humid-

ity was the same, but in the first case the temperature was 69°, while in the second it was 75°. The air moisture factor in the one case was 9.23 mm.; in the other, 12.65 mm. The relative humidity was the same, but the air moisture factors differed by 3.42 mm. Like relative humidities do not therefore present similar pictures of the air moisture condition except when the temperatures are the same. It is preferable, therefore, to determine the air moisture conditions from records of the evaporating power of the air as shown by a standard evaporating surface, such as the porous clay spheres, or Livingston atmometers.

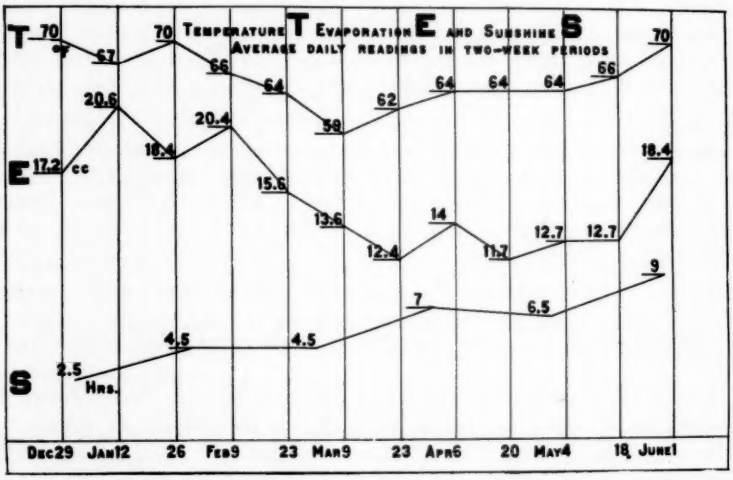

Fig. 3. Mean daily temperature, T, in degrees F.; mean daily evaporation, E, in cc.; and mean daily duration of sunshine, S, in hours in two-week periods. S is given for outdoors, and this condition is much reduced inside the greenhouse.

The air moisture condition, or atmometric index, was derived from records obtained from two standard white spherical atmometers kept on the rotating tables during the experiment. Humidity percentages were calculated three times daily (8 A. M., 12 M., and 6 P. M.) from results obtained by the sling psychrometer. The hygrograph did not prove satisfactory and was discarded. The sling psychrometer was mounted on a board, the bulb of the wet thermometer resting over a hole in the board, which allowed free circulation of air over the wet bulb. When a reading was desired, a current of air from an electric fan was directed at the apparatus, the reading made, and humidities calculated from the psychrometric tables. Curves

of temperature, evaporation, and sunshine duration are given in fig. 3. The results in table III are given in two-week periods.

Some mutual shading of the well-grown plants could not be avoided. An attempt was made, however, to minimize the error arising from this condition by changing the position of the cultures on the rotating table at the end of every three-day period, at the time when distilled water was added. Plants from the inner row were changed to the outer row and *vice versa*.

This consideration of the environment is by no means complete, nor is one to infer from the separate discussion of the individual factors already made that these act upon the plants singly. The influences acting upon the plant are the resultant of all factors. This is the reason why it has been so difficult to evaluate the climatic complex.

FUMIGATION OF GREENHOUSE.—On December 10th, just before the experiment was started, it was observed that mildew was appearing on wheat plants in other portions of the greenhouse. Fumigation was accomplished by painting the steam pipes that night with a sulphur paste. By carefully controlling the humidity conditions in the greenhouse, and by keeping the temperature near that suitable for the growth of wheat (60°–70° F. during the day, and 50°–60° F. during the night) we were not troubled again during the period of the experiment.

In early March thrips and lice appeared and these were controlled by fumigation over night with "Black Leaf 40" at the rate of one ounce for every 1,000 cubic feet of space. The fumigation was repeated the following morning. This controlled the insects for a time but they appeared again in April. On the 3rd of April, the third and last fumigation was made and this proved effective for the rest of the experimental period. These various fumigations had no deleterious effects on the wheat at any time.

This statement of the experimental methods, although too long, is desirable, for much of the difference in results obtained by various workers might be eliminated if the description of conditions were sufficiently detailed to allow real duplication of conditions.

Discussion of results

THREE SALT CULTURES

This experiment deals with the growth of Marquis wheat in soil (pot cultures) in the greenhouse during the winter of 1918–19. The plants were grown to maturity, were healthy and robust, and compared exceedingly well with plants grown in the field. The well known triangle system for determining the best salt ratio for optimum yields was tried out. The actual gain or loss in any particular culture is figured on the basis of check as unity, and is obtained by dividing the culture yield by the check yield.[1]

[1] The check yields are the yields from two cultures of twelve plants each, in fertile soil that had received no special fertilizer treatment.

The final results of the yield of grain and straw are given in tables IV and V. Special attention is called to column 8 in table IV which shows the average weight of grain relative to check as unity. It is from this column that a picture of the differences in yield for the various cultures can be drawn. The same is true for column 7 in table V in regard to straw yield. The last column in each table indicates the rank of the low and high yielding cultures. The nine high yielding cultures are indicated by an H preceded by the figure indicating the rank. The nine cultures showing the lowest yields are indicated by an L preceded by the figure indicating the rank.

The high and low yields are plotted on the triangle in figure 4. With very few exceptions, the low grain yields are grouped in that angle of the triangle which represents high ratios of K_2O. The same is true with respect to straw yields. High ratios of K_2O are not conducive to good growth. A further proof that high applications of K_2O are bad is found in the results obtained where this single salt is used. When 536 milligrams are added per pot (at the rate of 168 pounds per acre), the grain yield is reduced 7 per cent. and the straw yield by 18 per cent. when compared with the checks. When 268 milligrams are added per pot (application at the rate of 84 pounds per acre), the yield of grain is increased by 6 per cent. and the yield of straw by only 1 per cent. At the rate of 42 pounds per acre (134 milligrams per pot), the results are again negative, the grain yield being reduced 8 per cent. and the straw yield 2 per cent.

In regard to the high yields, the results as shown on the triangle are not what might have been expected. From *a priori* reasoning a single best culture should be found, and grading from this in all directions should be found the succeeding high yielding cultures. In this particular experiment there are two high points. Many workers have found two high regions and two low regions. No explanations have been offered, but it is evident that the centers of groups of good cultures might be just off the particular triangle studied, while a few cultures forming the outer margin of high yielding regions would enter the triangle. This explanation, however, is not adequate in the present case, as it is very apparent that three or four of the good cultures are scattered in widely different parts of the triangle with poor cultures their immediate neighbors.

A glance at the yield figures of tables IV and V shows one exceptionally high culture, and it could be argued that it gave an abnormally high yield, and should rightly be eliminated from consideration. Two or three other cultures could be eliminated for various reasons, but in this process of elimination where shall one stop? If we eliminate the cultures at the upper and lower right corners of the triangle, and the two in the medium lower right angle, there would be left five cultures of about the same grade of

TABLE IV

GRAIN YIELD DATA FOR MARQUIS WHEAT IN GREENHOUSE, DECEMBER 16, 1918–JUNE 9, 1919

CULTURE NUMBER	WEIGHT OF HEADS WITH GRAIN, ALL PLANTS	AVERAGE WEIGHT OF HEADS WITH GRAIN	TOTAL NUMBER OF GRAINS	AVERAGE NUMBER OF GRAINS PER PLANT	TOTAL WEIGHT OF GRAIN PER PLANT	AVERAGE WEIGHT OF GRAIN PER PLANT	AVERAGE WEIGHT OF GRAIN RELATIVE TO CHECK	PER CENT. OF GRAIN PER PLANT	WEIGHT OF 100 GRAINS	PER CENT. INCREASE GRAIN WEIGHT OVER CHECK	HIGH YIELDING AND LOW YIELDING CULTURES
	gm.	gm.			gm.	gm.	gm.		gm.	gm.	
1, 1, 8	11.1	0.925	243	20.3	8.50	0.708	1.20	76.5	3.50	20.0	
1, 2, 7	9.2	0.767	213	17.9	6.89	0.574	0.96	75.8	3.23	– 4.0	1L
1, 3, 6	9.1	0.758	194	16.2	7.00	0.583	0.98	76.9	3.61	– 2.0	2L
1, 4, 5	10.2	0.927	222	20.2	7.90	0.718	1.20	77.5	3.56	20.0	
1, 5, 4	9.2	0.767	196	16.3	7.01	0.584	0.98	76.1	3.58	– 2.0	3L
1, 6, 3	10.4	0.867	225	18.8	8.19	0.683	1.14	78.8	3.64	14.0	
1, 7, 2	11.3	0.942	237	19.8	8.80	0.733	1.23	77.8	3.71	23.0	9H
1, 8, 1	12.0	1.000	256	21.3	9.46	0.788	1.32	78.8	3.70	32.0	3H
2, 1, 7	9.2	0.836	203	18.5	7.10	0.645	1.08	77.2	3.50	8.0	
2, 2, 6	9.8	0.754	214	16.0	7.65	0.589	0.98	78.1	3.57	– 2.0	4L
2, 4, 4	10.3	0.858	229	19.1	8.09	0.674	1.13	78.6	3.53	13.0	
2, 5, 3	14.3	1.300	286	26.0	10.90	0.991	1.66	76.3	3.81	66.0	1H
2, 6, 2	10.2	0.850	225	18.8	7.99	0.666	1.11	78.4	3.55	11.0	
2, 7, 1	9.4	0.783	206	17.2	7.28	0.607	1.01	77.5	3.53	1.0	5L
3, 1, 6	8.9	0.809	199	18.1	7.01	0.637	1.06	78.7	3.52	6.0	
3, 2, 5	9.5	0.792	208	17.3	7.47	0.624	1.05	78.8	3.59	4.0	7L
3, 3, 4	9.8	0.817	216	18.0	7.47	0.624	1.04	76.4	3.46	4.0	8L
3, 4, 3	10.2	0.850	232	19.3	8.14	0.678	1.13	79.7	3.51	13.0	
3, 5, 2	11.1	0.925	229	19.1	8.49	0.708	1.18	76.5	3.70	18.0	
3, 6, 1	10.4	0.800	233	18.0	8.08	0.622	1.04	77.8	3.46	4.0	9L
4, 1, 5	12.7	1.058	265	22.1	9.82	0.818	1.37	77.3	3.72	37.0	2H
4, 2, 4	11.6	0.967	247	20.6	9.17	0.764	1.28	79.0	3.71	28.0	6H
4, 3, 3	11.2	0.933	242	20.2	9.01	0.751	1.26	80.5	3.72	26.0	7H
4, 4, 2	11.0	0.917	235	19.6	8.56	0.713	1.19	77.7	3.65	19.0	

TABLE IV—(*Continued*)

GRAIN YIELD DATA FOR MARQUIS WHEAT IN GRLENHOUSE, DECEMBER 16, 1918–JUNE 9, 1919

CULTURE NUMBER	WEIGHT OF HEADS WITH GRAIN, ALL PLANTS	AVERAGE WEIGHT OF HEADS WITH GRAIN	TOTAL NUMBER OF GRAINS	AVERAGE NUMBER OF GRAINS PER PLANT	TOTAL WEIGHT OF GRAIN PER PLANT	AVERAGE WEIGHT OF GRAIN PER PLANT	AVERAGE WEIGHT OF GRAIN RELATIVE TO CHECK	PER CENT. OF GRAIN PER PLANT	WEIGHT OF 100 GRAINS	PER CENT. INCREASE GRAIN WEIGHT OVER CHECK	HIGH YIELDING AND LOW YIELDING CULTURES
	gm.	gm.			gm.	gm.	gm.		gm.	gm.	
4, 5, 1	10.2	0.850	213	17.8	7.79	0.649	1.09	76.4	3.66	9.0	
5, 1, 4	9.6	0.800	203	16.9	7.29	0.608	1.02	76.0	3.59	2.0	6L
5, 2, 3	10.5	0.875	230	19.2	8.13	0.678	1.13	77.5	3.53	13.0	
5, 3, 2	11.4	0.950	234	19.5	8.78	0.732	1.23	77.1	3.75	23.0	8H
5, 4, 1	10.7	0.892	224	18.7	8.38	0.698	1.17	78.2	3.74	17.0	
6, 1, 3	10.2	0.850	218	18.2	7.94	0.662	1.11	77.9	3.64	11.0	
6, 2, 2	11.7	0.975	250	20.8	9.13	0.761	1.28	78.1	3.65	28.0	5H
6, 3, 1	10.1	0.842	226	18.9	7.87	0.656	1.09	77.9	3.48	9.0	
7, 1, 2	10.5	0.875	227	18.9	8.19	0.683	1.14	78.1	3.61	14.0	
7, 2, 1	10.6	0.883	234	19.5	8.23	0.686	1.15	77.7	3.52	15.0	
8, 1, 1	12.0	1.000	250	20.8	9.28	0.773	1.29	77.3	3.71	29.0	4H
Check 1	9.7	0.808	208	16.9	7.65	0.000	0.00	00.0	0.00	0.0	
Check 2	8.9	0.742	197	16.4	6.82	0.598	1.00	77.4	3.71	0.0	
P_2O_5 1	10.4	0.867	233	19.5	8.21	0.684	1.14	78.9	3.52	14.0	
P_2O_5 2	10.0	0.769	215	16.6	7.71	0.593	0.99	77.1	3.59	− 1.0	
P_2O_5 3	10.0	0.833	215	17.9	7.79	0.649	1.09	77.9	3.62	9.0	
P_2O_5 4	8.4	0.700	179	14.9	6.43	0.536	0.90	76.6	3.59	−10.0	
N 1	11.5	0.959	236	19.7	8.92	0.743	1.24	77.5	3.78	24.0	
N 2	12.6	1.050	266	22.2	9.91	0.826	1.38	78.7	3.73	38.0	
N 3	8.5	0.709	188	15.7	6.52	0.543	0.91	76.6	3.47	− 9.0	
K_2O 1	8.7	0.725	189	15.8	6.66	0.555	0.93	76.6	3.54	− 7.0	
K_2O 2	10.5	0.808	230	17.7	8.23	0.633	1.06	78.3	3.58	6.0	
K_2O 3	8.5	0.654	189	14.6	6.56	0.547	0.92	83.7	3.47	− 8.0	

TABLE V

STRAW YIELD DATA FOR MARQUIS WHEAT IN THE GREENHOUSE, DECEMBER 16, 1918–JUNE 9, 1919

CULTURE NUMBER	AVERAGE LENGTH OF PLANTS	AVERAGE LENGTH OF HEADS	AVERAGE LENGTH OF STRAW	WEIGHT OF STRAW ALL PLANTS	AVERAGE WEIGHT OF STRAW PER PLANT	WEIGHT OF STRAW RELATIVE TO CHECKS	PER CENT. INCREASE OR DECREASE OVER CHECK	HIGH YIELDING AND LOW YIELDING CULTURES
	cm.	cm.	cm.	gm.	gm.	gm.		
1, 1, 8	104.3	7.3	97.0	17.2	1.43	1.13	13.0	
1, 2, 7	95.3	7.3	88.0	17.3	1.19	0.94	– 6.0	1L
1, 3, 6	103.1	6.9	96.2	15.3	1.28	1.02	2.0	7L
1, 4, 5	106.3	7.6	98.7	16.6	1.51	1.19	19.0	
1, 5, 4	98.6	7.3	91.3	14.4	1.20	0.95	– 5.0	3L
1, 6, 3	104.3	8.1	96.2	16.9	1.41	1.12	12.0	
1, 7, 2	99.6	7.5	92.1	16.6	1.38	1.09	9.0	
1, 8, 1	109.2	8.5	100.7	19.4	1.62	1.28	28.0	6H
2, 1, 7	102.1	8.6	93.5	16.4	1.49	1.18	18.0	
2, 2, 6	103.3	7.1	96.2	15.5	1.19	0.94	– 6.0	2L
2, 4, 4	105.6	8.3	97.3	16.6	1.38	1.09	9.0	1H
2, 5, 3	114.1	9.5	104.6	21.1	1.92	1.52	52.0	
2, 6, 2	106.4	8.4	98.0	18.0	1.50	1.19	19.0	
2, 7, 1	104.2	7.8	96.4	16.6	1.38	1.09	9.0	5L
3, 1, 6	103.9	7.0	96.9	13.9	1.26	1.00	0.0	4L
3, 2, 5	103.8	6.8	97.0	14.6	1.22	0.97	– 3.0	
3, 3, 4	102.8	8.0	93.8	16.7	1.39	1.09	9.0	
3, 4, 3	108.2	7.5	100.7	16.8	1.40	1.09	9.0	
3, 5, 2	110.7	9.1	101.6	18.3	1.53	1.21	21.0	
3, 6, 1	105.7	8.9	96.8	18.8	1.45	1.15	15.0	2H
4, 1, 5	111.0	8.3	102.7	20.5	1.71	1.36	36.0	8H
4, 2, 4	111.4	8.1	103.3	18.7	1.56	1.24	24.0	
4, 3, 3	113.7	7.7	106.0	19.7	1.64	1.30	30.0	4H
4, 4, 2	107.9	8.4	99.5	17.6	1.47	1.17	17.0	

TABLE V—(*Continued*)

STRAW YIELD DATA FOR MARQUIS WHEAT IN THE GREENHOUSE, DECEMBER 16, 1918–JUNE 9, 1919

CULTURE NUMBER	AVERAGE LENGTH OF PLANTS	AVERAGE LENGTH OF HEADS	AVERAGE LENGTH OF STRAW	WEIGHT OF STRAW ALL PLANTS	AVERAGE WEIGHT OF STRAW PER PLANT	WEIGHT OF STRAW RELATIVE TO CHECKS	PER CENT. INCREASE OR DECREASE OVER CHECK	HIGH YIELDING AND LOW YIELDING CULTURES
	cm.	cm.	cm.	gm.	gm.	gm.		
4, 5, 1	107.6	8.7	98.9	17.1	1.43	1.14	14.0	
5, 1, 4	104.3	6.9	97.4	15.3	1.28	1.02	2.0	6L
5, 2, 3	109.6	8.6	101.0	18.2	1.52	1.21	21.0	9H
5, 3, 2	109.6	8.8	100.8	20.1	1.68	1.33	33.0	3H
5, 4, 1	106.9	8.0	98.9	17.7	1.48	1.17	17.0	
6, 1, 3	106.9	7.8	99.1	16.5	1.38	1.09	9.0	
6, 2, 2	111.7	8.0	103.7	19.5	1.63	1.30	30.0	5H
6, 3, 1	109.6	7.4	102.2	15.8	1.32	1.05	5.0	8L
7, 1, 2	105.6	7.9	97.7	16.5	1.38	1.09	9.0	
7, 2, 1	104.6	8.5	96.1	16.2	1.35	1.07	7.0	9L
8, 1, 1	109.9	8.7	101.2	19.3	1.61	1.28	28.0	
Check 1	106.4	7.4	98.0	15.7	1.32	1.00	0.0	7H
Check 2	100.5	7.7	92.8	14.4	1.20	1.00	0.0	
P_2O_5 1	104.9	7.8	97.1	16.5	1.37	1.09	9.0	
P_2O_5 2	112.4	7.9	104.5	15.6	1.20	0.95	− 5.0	
P_2O_5 3	104.6	9.1	95.5	15.4	1.28	1.02	2.0	
P_2O_5 4	96.6	6.8	89.8	12.9	1.08	0.86	−14.0	
N 1	106.7	8.4	98.3	17.6	1.48	1.18	18.0	
N 2	107.0	8.3	98.7	17.9	1.49	1.19	19.0	
N 3	100.6	7.3	93.3	13.0	1.08	0.86	−14.0	
K_2O 1	98.3	7.2	91.1	12.4	1.03	0.82	−18.0	
K_2O 2	102.9	8.4	94.5	16.5	1.27	1.01	1.0	
K_2O 3	99.3	7.1	92.2	13.1	1.01	0.80	−20.0	

value near the upper middle region. This shows that there is a demand for acid phosphate, and is in accord with the general field experience that small grains require considerable phosphoric acid. Perhaps it may be safe to say that phosphoric acid is required more than nitrogen since there are more high cultures nearer that angle than the nitrogen angle. This reasoning, however, is not satisfying.

It is not beyond reason to try to trace the condition found to variability of the individual plants. It has been suggested that variability of plants in water culture is so great that it may obscure the relation between the growth of plants and the physiological values of the solutions tested. The writer has demonstrated this in his own experiments several times in the last six or eight years. The same might be true of plants in soil cultures, but it is not so strikingly evident in this experiment. The seeds used were from a pure line; they were selected carefully according to standard rules, were germinated under controlled conditions, and the test plants selected for uniform height and growth from a large group. All were planted at the same depth in the soil and given the same care. At harvest time, the height as well as the length of head of every plant in each culture was measured. In addition, the following data were also collected for each culture: Total weight of heads and grain, total weight of grain, total number of grains, weight of 100 grains, and total weight of straw.

Variability in the height of the plants in a culture was quite small. The coefficients of variability for all of the cultures were determined and the average was 8.2 per cent. The highest coefficient in the whole group was 13.3 per cent. This is not considered high, judging from the records which have been obtained from various experiments both in the greenhouse and in the field under conditions where great variability was sought, and where, by controlling conditions, variability could be held down to a low per cent. There was considerable variability between cultures; but within a single culture there was very little individual variability. By further study it was found that among the 9 best cultures there was a real difference only when the best and the poorest in the group were compared. The odds of 144 to 1 showed a real difference between the poorest and the best in the group of 9 best cultures. The others did not show any significant difference. LOVE's modification of Student's Method was used in these computations. When any culture in the group of 9 best was compared with any in the group of the 9 poorest cultures, the differences were significant also. Finally as to the check cultures, two in number, it can be said that they were similar, there being no significant differences between them as far as the height of the plants is concerned. Calculated by Student's method, the odds were merely 10.9 to 1 and by BESSEL's method, 13.5 to 1. The above facts indicate that variability within a culture is not sufficiently large to

explain the lack of satisfactory grouping of the high yields on the triangle. Furthermore, the data show that the salt ratios produced differences in cultures throughout the triangle. The relation between the growth of plants and the physiological values of the solutions were not obscured, therefore, by variability. To charge the lack of grouping to variability in seedlings seems begging the question.

These considerations are based on records of the height of individual plants. That conclusions drawn from these height measurements are as reliable as those from individual dry weights or grain weights, which are more often offered, can be shown. The individual grain weight records were not gathered in this particular experiment, but those of the culture as a whole (12 plants) were obtained.

TABLE VI

CORRELATION BETWEEN HEIGHT OF PLANTS AND DRY WEIGHTS

Length of plants in cm., average of 12 plants

Weight of grain, average of 12 plants	96	99	100	102	103	104	105	107	109	111	112	114
6.5	1	2	1									
7.0	1	1	1	1	1	1						
7.5				1	1	3	1					
8.0					1	3	4	4	3			
8.5						1		2		1		
9.0								1	1	2		1
9.5									2			
10.0								1		1		
11.0												1

The correlation between height of plants and weight of grain is shown in table VI. It shows a high positive correlation between the height of plants per culture and the weight of grain per culture. It is perfectly logical, therefore, to draw the conclusion that height of individual plants is correlated with their yield, and that the argument previously developed is just as important as if it had been based entirely on individual grain yields instead of height of individuals.

SCHREINER and SKINNER, who were originally responsible for the use of the triangular system for determining the salt requirements of agricultural crops in water cultures, early concluded that the ratios of salts might vary widely without appreciably affecting the yields. Others, working more

recently, though not using the triangular system in detail, have drawn the same conclusion, and might use the data here presented to support their own conclusions. Perhaps they would be correct. There is, however, another suggestion that might be presented and which may have more than a slight bearing on the question. Our agricultural crops have been for generations so modified by domestication that they have become accustomed to wide variation in environmental conditions and do not lend themselves to studies of this kind. Results might be very different with some wild variety raised in a restricted environment.

Finally, the observed facts may be due to the possible variability in the soil. The soil is a very complex medium for the growth of plants. In the field, there are many differences in localities within a few feet of each other. In this particular experiment every effort was made to prepare the soil so that uniform samples could be obtained for each culture. The soil was first air dried, then sifted, then redried, and broken up finally to pass through a 4 mm. mesh sieve. It was then stored in tight cans until needed. When ready to prepare the cultures, a definite amount of soil for each culture pot (6,400 grams) was placed on a clean paper on the table and thoroughly mixed with the sample of fertilizer for that particular culture. This mixing was done according to the plan suggested by TRUOG (**14**). It is not believed that the differences in the cultures were due to any possible difference in the soil used. That the differences are attributable to the fertilizer ratios is more logical. That the poor grouping of the best cultures could be traced to the soil used is not likely, considering the great care taken to make the soil uniform for all culture pots.

A duplicate of this experiment was started in September and was completed in March. There was excellent vegetation growth, but no heading out even after a period of one hundred and sixty-eight days. Light was considered the limiting factor, during the early period at least. After a careful study of the cultures as to general appearance, it was found that the same general conclusions would have to be drawn as in the first case. The experiment was terminated with no further collection of dry weight or other data.

In addition to the method illustrated in figure 4, there is still another way of calculating the effects of the fertilizer salts, *viz.*: by arranging the various cultures in groups. For example (see figure 5), the ten cultures in the top portion of the triangle represent cultures high in acid phosphate, while a similar number of cultures in the lower right portion represents cultures high in sodium nitrate. In the lower left portion of the triangle, the ten cultures are high in potassium sulphate. In the center of the triangle there are six cultures in which all three fertilizer ingredients are in equal or nearly equal amounts. When the average grain and straw weights

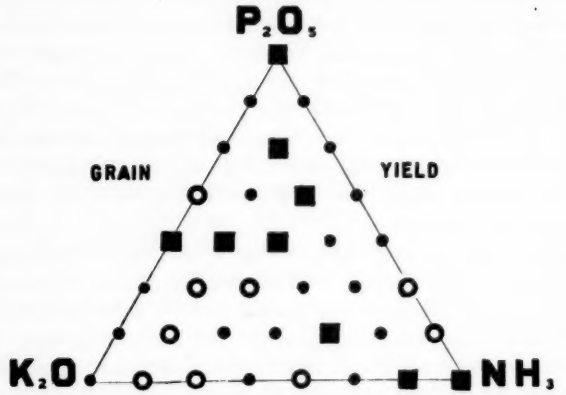

FIG. 4. Location of nine best cultures, ■ and nine poorest cultures, o. Data from the tables.

of these various regions or groups are calculated in terms of increase over check and then compared, no significant differences are found except in the case of cultures in the groups high in K_2O. The figures of the three groups are almost identical. This bears out the observation that the good cultures are scattered. When, however, the value of the group high in potassium sulphate was calculated, it was found to be considerably lower than the other

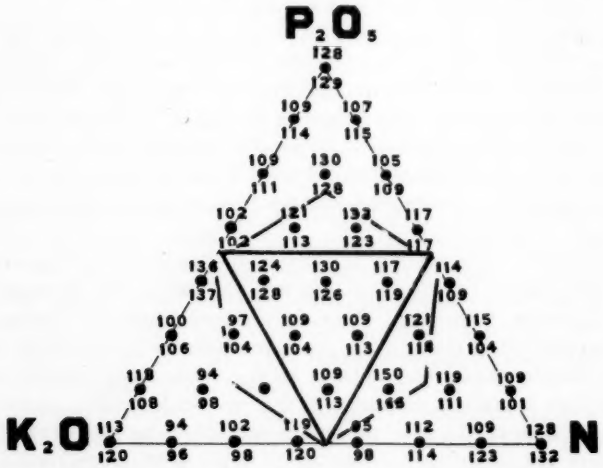

FIG. 5. A method of grouping by cultures. See text for explanation.

three, and showed that the poor cultures were well clustered in one region of the triangle.

The figures for grain yield are: Acid phosphate 16.1 per cent., sodium nitrate 17.6 per cent., center of the triangle 17.1 per cent., and potassium sulphate 9.6 per cent. The same order was followed when the straw yields were taken and calculated in like manner. This method does not give us results differing in any appreciable way from the other methods mentioned. This is a method that has been used by HARRIS (**2**), and in a modified form by WOODS (**15**).

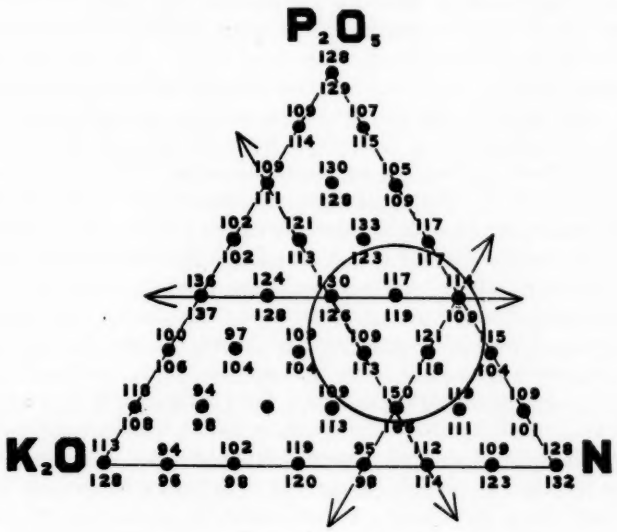

FIG. 6. Another method of grouping.

Finally, in figure 6 there is illustrated another method which shows that the concentration of the three fertilizer ingredients can vary quite a little and still not modify the yield to any appreciable extent. This method has been suggested by SCHREINER and SKINNER in the work referred to in the earlier part of this paper. In this calculation, the records for the cultures at the angle at the top, and that in the angle at the lower right were eliminated. This method in our opinion is no better than the others already discussed when one attempts to interpret the results.

It seems impossible to ascribe the lack of grouping of the best cultures to any other reason than that the plants used are accustomed to a variety of salt ratios and do not suffer appreciable differences in yield.

SINGLE SALT CULTURE

On referring to table I, the amounts of single fertilizer compounds per pot and per acre can be seen. At the end of tables IV and V are given the data for grain and straw yields from plants grown in pot cultures containing the amounts of the single salts indicated. Taken as a whole, the use of single fertilizers does not give as good results in soil cultures as the combination of three under the conditions of this experiment. Several other similar experiments have shown the same results. This had been previously shown for water cultures, and is now shown to be true for soil cultures even though such soil samples were from a field in high fertility. Of the ten single salt cultures, only two reached the value of the high group and these were the cultures where nitrogen was used alone. The per cent. gain in grain yield over the checks was 24 with an application of 1.696 gram per pot or on the basis of 528 pounds per acre; with an application of 0.848 gram per pot, equivalent to 264 pounds per acre, the gain was 38 per cent. The latter rate of application was better than the former as far as grain yield was concerned. The application of nitrogen benefited grain development, but vegetative growth as shown by straw yield did not equal any of the best nine cultures in the group containing the three fertilizer ingredients. As for $Ca(H_2PO_4)_2$ the best results obtained with four different rates of application was only 9 per cent. better than the checks. In this case the amount applied was 1.552 gram per pot, equal to 480 pounds per acre. The other three applications were at decreasing rates, the results showing inferiority to the yields of the controls. The yields of grain and straw when K_2SO_4 was applied at three different rates were either inferior to the controls, or equal to them, but not better. An application at the rate of 0.536 gram per pot, or 168 pounds per acre, was detrimental, cutting down the yield of both straw and grain. At one-fourth the application the yield was subnormal, while at one-half it was about the same as that of the controls.

Although the best yields are obtained with this soil where the three fertilizers are mixed, the increased cost of the mixture might make its use prohibitive. Only under such conditions would it be advisable to resort to the use of a single fertilizer. To determine whether a triple mixture with its increased yield over that of a single fertilizer costs less in the long run, a regular field experiment should be inaugurated.

CONDITION OF PLANTS

For the early period of growth the plants were normal in all respects and except for size there were no apparent differences between the cultures. The normal plant out in the field averages two or three tillers, while those grown in the greenhouse tillered sparingly. This was the only difference between the field and the greenhouse cultures. Only sixteen out of the

thirty-five different cultures showed tillers and none of these headed out. Culture 1, 1, 8, only an average yielder, produced 5 tillers. Only one culture (3, 3, 4), and this a poor yielder, possessed 4 tillers. There were four cultures that had three tillers each (1, 2, 7; 2, 1, 7; 2, 5, 3; and 6, 3, 1). All were poor yielders except 2, 5, 3, which was the best. The cultures possessing 2 tillers were two in number (2, 6, 2 and 4, 4, 2), merely average yielding cultures. There were eight cultures showing one tiller (1, 3, 6; 1, 5, 4; 1, 8, 1; 2, 4, 4; 3, 4, 3; 3, 6, 1; 5, 4, 1; and 8, 1, 1) varying from poor yielding cultures, through medium, to very high yielding ones. Under the conditions of this experiment one would not be justified in concluding that fertilizer combinations were even contributory agents in the formation of tillers.

On April 11 there was the first appearance of heads in cultures 3, 5, 2 and 6, 3, 1. On the 14th, heads appeared on cultures 1, 8, 1; 2, 1, 7; 1, 4, 5; and 8, 1, 1. On the 18th all the cultures with the exception of 1, 2, 7 and 1, 3, 6 had one or more plants showing heads. At this time out of a possible 418 plants there were 157 showing heads (37.5 per cent.). Five days later out of a possible 418 plants there were 363 showing heads (approximately 80 per cent.). The rest headed out a few days later. In approximately two weeks from the first appearance of a head they all headed. Blossoming started on April 11th and continued through and slightly beyond the period of heading out. The plants were harvested on the 9th of June after a growing period of 174 days. A good idea of the general appearance (color excepted) of the plants may be judged from the photographs, figures 1 and 2.

The plant measurements taken at harvest time, but not given here, comprise the following: Height of plant, length of head, and number of heads.

Transpiration

All the transpiration data include not only the loss of water from plants but also that from the surface soil in the pots. These pot cultures were not sealed with wax as is often done for the purpose of eliminating all moisture losses from the soil. To obtain an idea of the amount thus lost, two crocks of soil without plants were run along with the others as a check. When the other cultures received a gravel mulch, these two also received the same. A number of experiments showed that the loss of water by evaporation from the soil was very small and the water loss from the cultures may be assigned to water loss from the plants. Leathers in India (**6**) and others have also found that the water loss from soil was very little and could be neglected from calculation, or determined from control pots and the percentages calculated.

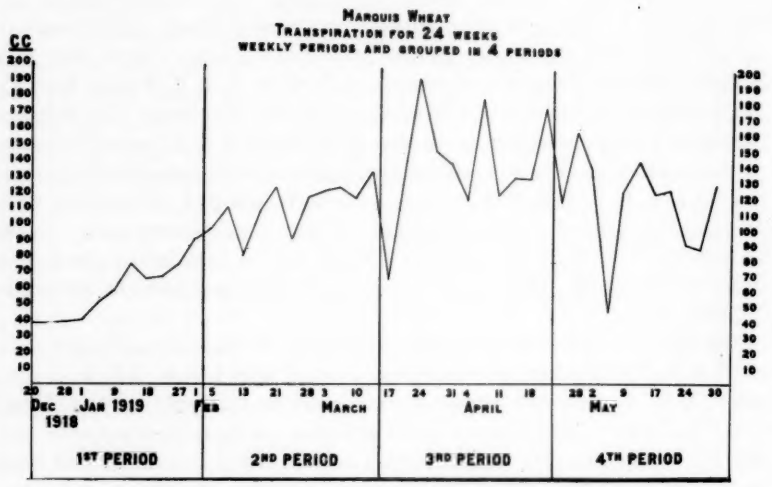

FIG. 7. Mean daily transpiration of all plants throughout the period of growth, plotted by weeks, and divided into four periods.

Transpiration was determined by weight which is more accurate than volume determinations. The first weighings were made on December 20th and every 3.5 days thereafter until June 3rd. Figure 7 is a curve showing the loss of water from soil and plants for the period of December 20 to May 30. The entire period of 161 days has been divided into four smaller periods of five to six weeks duration for the purposes of comparison. It will be observed that the loss at the start is small and this increases gradually until it reaches a maximum at the latter part of March, just a short time before the heading out of the grain, and then it falls off to the last day. The curve resembles a growth curve in that there is a grand period of water loss like the "grand period of growth." During the heading out period, the greatest amount of water is called for, and transpiration is greatest at this time. It suggests that transpiration may be a good measure of plant growth. Curves for mean daily transpiration and evaporation ratio for two-week periods are shown in figure 8. The transpiration data are not given.

WATER REQUIREMENT

The amount of water loss for each culture for every 3.5 day period was determined, but the data are not given here. Since the water requirement

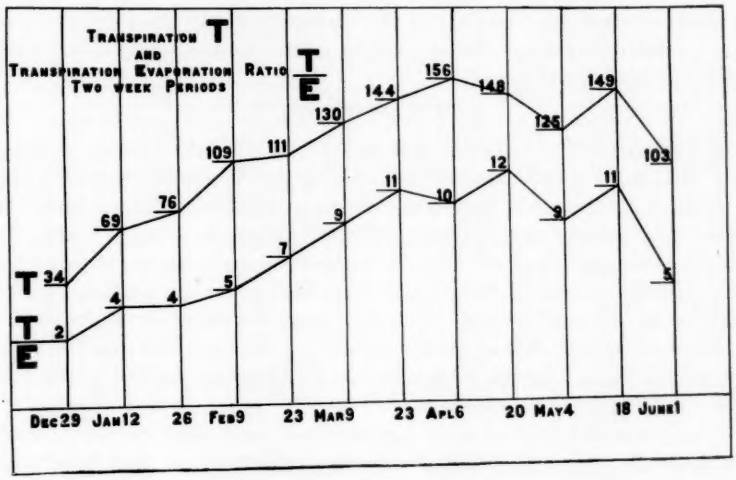

FIG. 8. Mean daily transpiration T, in cc. for two-week periods. Mean daily transpiration-evaporation ratio, $\frac{T}{E}$, calculated from cc. in two-week periods.

is the ratio between the amounts of water lost by transpiration and the dry weight yield, it may be used as a criterion of growth. The interesting feature of the water requirement data is the evidence that the high yielding cultures have a lower water requirement than any of the other cultures (1, 7, 2; 1, 8, 1; 2, 5, 3; 4, 1, 5; 4, 2, 4; 4, 3, 3; 5, 3, 2; 6, 2, 2; and 8, 1, 1). The highest yielding nine cultures have a water requirement of approximately 600 or less, while the remaining cultures are above this. The best salt proportions of the fertilizer ingredients apparently give a low water requirement, and the poorer salt proportions give the highest. Not only is it true that increasing amounts of the fertilizer mixture, up to a certain limit, lowers the water requirement, but also a change to the proper ratio of the fertilizer ingredient seems to decrease the water requirement.

The experiment was so planned that the data could be subjected to statistical treatment. There were twelve plants in each of the two control pots, providing data on 24 plants to be used for comparison with the 12 plants in each of the other cultures. Student's method and BESSEL'S were used in making these comparisons and significant differences were found, so that the highest yielding cultures fell in one group and the lowest yielding cultures fell in another group just as can be shown by the actual results of yields, etc.

However, this sort of statistical treatment is not necessary in the present work where an explanation of the scattering of the good cultures over the triangular diagram is under consideration. Consequently a discussion of these results is omitted.

Field experiment

The experiment reported in this section was our first attempt at applying a wide range of salt proportions in the field. The three fertilizers commonly used in a complete fertilizer mixture, calcium acid phosphate, sodium nitrate, and potassium sulphate, were the compounds or salts used. The soil was taken just as it was without a study of its chemical, physical and biological characteristics, and treated with the various proportions or combinations of fertilizer salts. The influence of the fertilizer is judged according to the effect it has on the growth of oats taken as the indicator. This same general plan has been followed for three successive years following the date of this experiment.

It was expected that many of the details of the method employed would have to be changed or modified as experience dictated, and that some methods would have to be discarded. The experiment was merely a preliminary one and many suggestions for guidance in future work have come out of it.

There was no special preparation of this small strip of level land, approximately 500 feet long and 60 feet wide, which was set aside for this experiment. The crop of the preceding year was beans. Early in the following spring, manure was spread on, and plowed under shortly afterwards. It was plowed in the usual way and then the seed bed was prepared for the drilling of oats. Worthy oats obtained from the Farm Crops Department were first treated with formaldehyde for smut and then planted at the rate of six pecks to the acre. Planting was done on May 8th, the rows running the long way of the field. The weather was good and the stand excellent.

A couple of days before the fertilizer was applied, the ground was staked out. The plan of series C is shown in fig. 9; series B and A, not shown in the figure, are continuous towards the east. Each series is separated from its neighbor by an alley five feet wide running across the rows. There were forty-four plots in each series, each plot being forty feet long and two feet wide. Each plot is made up of four rows of plants, the two center ones being taken as the experimental rows. The fertilizer was distributed between these on June 5th. At this time the plants were six to eight inches tall. Several border rows bounded the strip of land on sides and ends. No check or fertilizer plot was placed on the dead furrow. Before harvesting, the two outer rows in each plot were pulled up and this aided considerably in marking off clearly the fertilized and check rows so that harvesting could be quickly done. The check and fertilized rows were hand cut August 9th, and the rest machine cut at a later date.

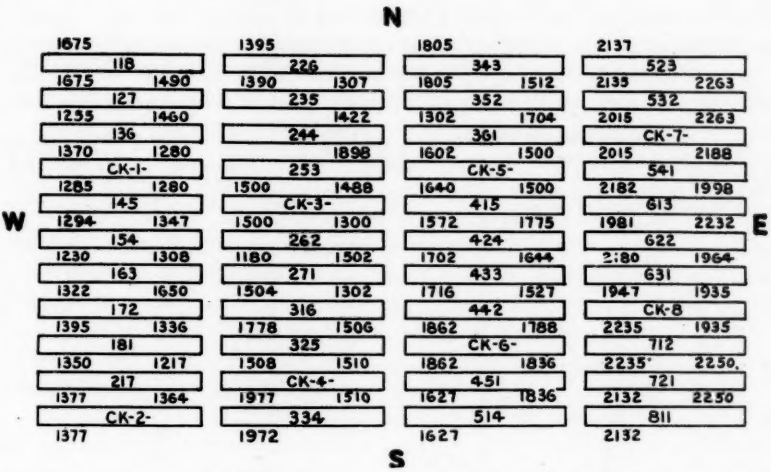

FIG. 9. Arrangement of field plots, series C. Numbers in rectangles show proportions of fertilizers. See text for details.

In fig. 9, the numbers in the rectangular blocks represent the proportions of the three salts, distributed on the basis of ten per cent. increments. The first number stands for calcium acid phosphate, the second for sodium nitrate, and the third for potassium sulphate. The fertilizer mixture for plot 5, 2, 3 then contains 5 parts of calcium acid phosphate, 2 parts of

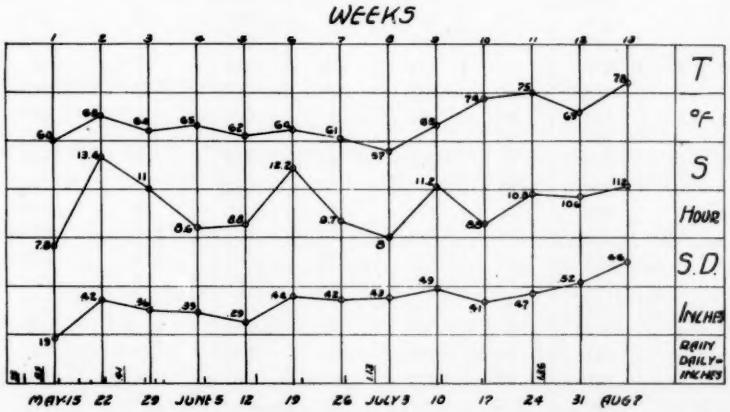

FIG. 0. Mean daily temperature, T, in °F.; mean daily sunshine, S, in hours; mean daily saturation deficit, S.D., in inches; and daily rainfall in inches, for each week of season.

TABLE VII

QUANTITIES OF FERTILIZER, AND ACTIVE INGREDIENTS PER PLOT. TOTAL AMOUNT OF FERTILIZER PER PLOT, 2 POUNDS (1089 POUNDS PER ACRE) IN 10 PER CENT. STAGES

PLOT NUMBER	ACID PHOSPHATE 16.5 PER CENT P_2O_5		SODIUM NITRATE 15.1 PER CENT. N		POTASSIUM SULPHATE 47.79 PER CENT. K_2O		TOTAL ACTIVE INGREDIENTS
	Fertilizer	Active ingredient	Fertilizer	Active ingredient	Fertilizer	Active ingredient	P, N, K
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1, 1, 8	91	15	91	14	726	347	376
1, 2, 7	91	15	181	27	635	303	345
1, 3, 6	91	15	272	41	544	260	316
1, 4, 5	91	15	363	55	454	217	287
1, 5, 4	91	15	454	69	363	173	257
1, 6, 3	91	15	544	82	272	129	226
1, 7, 2	91	15	635	96	181	87	198
1, 8, 1	91	15	726	110	91	53	178
2, 1, 7	181	30	91	14	635	303	347
2, 2, 6	181	30	181	27	544	260	317
2, 3, 5	181	30	272	41	454	217	288
2, 4, 4	181	30	363	55	363	173	258
2, 5, 3	181	30	454	69	272	129	228
2, 6, 2	181	30	544	82	181	87	199
2, 7, 1	181	30	635	96	91	53	179
3, 1, 6	272	45	91	14	544	260	319
3, 2, 5	272	45	181	27	454	217	289
3, 3, 4	272	45	272	41	363	173	259
3, 4, 3	272	45	363	55	272	129	229
3, 5, 2	272	45	454	69	181	87	201
3, 6, 1	272	45	544	82	91	53	180
4, 1, 5	363	60	91	14	454	217	291
4, 2, 4	363	60	181	27	363	173	260
4, 3, 3	363	60	272	41	272	129	230
4, 4, 2	363	60	363	55	181	87	202
4, 5, 1	363	60	454	69	91	58	182
5, 1, 4	454	75	91	14	363	173	262
5, 2, 3	454	75	181	27	272	129	231
5, 3, 2	454	75	272	41	181	87	203
5, 4, 1	454	75	363	55	91	53	183
6, 1, 3	544	90	91	14	272	129	233
6, 2, 2	544	90	181	28	181	87	204
6, 3, 1	544	90	272	41	91	53	184
7, 1, 2	635	105	91	14	181	87	206
7, 2, 1	635	105	181	27	91	53	185
8, 1, 1	726	120	91	14	91	53	187

sodium nitrate and 3 parts of potassium sulphate. This makes a total of ten parts of all the ingredients. The proportions for all the other fertilized plots can be determined in like manner. One plot in each of the first, third, and fourth rows was set aside for single fertilizer applications.

The application of fertilizer was at the rate of 2 pounds to the plot in all cases. The amount of active ingredient was much less since the fertilizer contained a filler. The amount of fertilizer and active ingredients per pot in grams is found in table VII. It will be noted from this table that the individual ingredients vary progressively, but that the total concentration of fertilizer in each plot is not the same. In the case of water cultures strict attention is given to this feature so that all cultures have the same total concentration. In regard to soil plots in the field, the concentration cannot be controlled, for it differs at different times under the varying climatic and edaphic conditions.

All these various fertilizer combinations were prepared beforehand and put into separate paper sacks, clearly labelled. They were then taken to the field and placed on their respective plots to avoid any chances of mistake. One person spread the entire set of combinations for the three series, this being deemed necessary for uniform distribution. After a little practice these various fertilizers can be uniformly spread. A light rain fell the evening after the fertilizer was spread. Two days later a heavier rain fell and helped considerably to incorporate the fertilizer with the soil. The field was kept fairly free from weeds by hoeing. When the plants became large enough to shade the growth beneath, weeding became necessary. The stand of oats was an excellent one and the climatic conditions were especially conducive to good growth during the season.

Environmental conditions

Tables follow showing the temperature, rainfall, saturation deficit, and sunshine data effective during growth. The data from which these tables were made were kindly given us by Mr. D. A. Seeley, of the local Weather Bureau.

In the form usually given the data do not represent exact conditions, for the means or averages give an inadequate idea of the influence of the various climatic conditions on the plant. Obviously, the intensity and duration of the extremes of the various factors play a larger rôle in the plant's development than if the plant were exposed for a long period at some point between the extremes. This may be illustrated in the following manner: There is a certain stage in the life of the oat plant when water is needed more than at another time. This is the critical period as far as water is concerned. During the first ten days after blossoming the

plant should have an abundance of moisture to help fill out the grain and bring the head to perfection. If moisture is lacking at this time the result is consequently poor grain. In a period like this, abundant moisture is not a detriment, but if the temperature is high and the season dry, deleterious effects are produced. The present season had no extremes that held for long periods. This may be observed by a study of the table.

In table VIII, the mean daily temperature, sunshine, saturation deficit and rainfall are recorded in weekly periods. Temperature is given in degrees F., sunshine in hours, saturation deficit and the rainfall, in inches. The conditions are shown graphically in fig. 10.

TABLE VIII

MEAN DAILY TEMPERATURE T, SUNSHINE S, SATURATION DEFICIT S.D., AND RAINFALL R, RECORDED IN WEEKLY PERIODS

WEEKLY PERIODS	TEMPERATURE	SUNSHINE	SATURATION DEFICIT	RAINFALL
	°F.	hours	inches	inches
May 8–May 15	60	7.8	0.19	0.20
May 16–May 22	68	13.4	0.42	0.03
May 23–May 29	64	11.0	0.36	0.10
May 30–June 5	65	8.6	0.35	0.01
June 6–June 12	62	8.8	0.29	0.02
June 13–June 19	64	12.2	0.44	0.03
June 20–June 26	61	9.7	0.42	0.03
June 27–July 3	57	8.0	0.43	0.22
July 4–July 10	65	11.2	0.49	Trace
July 11–July 17	74	8.8	0.41	0.08
July 18–July 24	75	10.8	0.47	Trace
July 25–July 31	69	10.6	0.52	0.20
August 1–August 7	78	11.2	0.66	0.01

The saturation deficit for the week is obtained by averaging the daily saturation deficits. The daily saturation deficits are derived from the noon temperatures and the vapor pressures for that day. The vapor pressure is found in the Weather Bureau psychrometric tables. When the observed vapor pressure for any particular day is subtracted from the vapor pressure at saturation for that day the saturation deficit is obtained. It is believed that this gives a better criterion of the moisture condition of the air than relative humidity and for this reason the method has been employed (**11, 8**).

Discussion of results

As is the usual custom, grain yield is taken here as the criterion in determining the influence of the different salt combinations. When the plants

TABLE IX

ACTUAL YIELDS AND RELATIVE WEIGHTS OF GRAIN OF OATS GROWN IN THE FIELD FROM MAY 8TH TO AUGUST 9TH. EXPERIMENT RUN IN TRIPLICATE, A, B, C

PLOT NUMBER	YIELD			YIELD RATIO TO CHECK AS UNITY			
	A	B	C	A	B	C	AVERAGE
	gm.	gm.	gm.				
1, 1, 8	1600	1220	1675	1.00	0.83	1.03	0.95
1, 2, 7	.....	1298	1490	.....	0.88	0.92	0.90
1, 3, 6	1325	1310	1255	0.83	0.87	0.77	0.83
1, 4, 5	1527	1372	1285	0.96	0.92	0.79	0.89
1, 5, 4	1262	1255	1347	0.79	0.85	0.83	0.82
1, 6, 3	1235	1325	1230	0.77	0.90	0.76	0.81
1, 7, 2	1343	1212	1650	0.84	0.83	1.01	0.89
1, 8, 1	1200	1635	1395	0.75	1.10	0.86	0.90
2, 1, 7	1410	1590	1217	0.88	1.07	0.75	0.90
2, 2, 6	2050	.....	1395	1.29	.....	0.86	1.07
2, 3, 5	2100	1235	1397	1.32	0.84	0.81	0.99
2, 4, 4	1625	1390	.....	1.02	0.94	.....	0.98
2, 5, 3	1450	1368	1898	0.91	0.92	1.17	1.00
2, 6, 2	1675	1050	1300	1.05	0.71	0.60	0.85
2, 7, 1	1637	1387	1180	1.03	0.94	0.73	0.90
3, 1, 6	1530	1245	1312	0.96	0.78	0.79	0.84
3, 2, 5	1300	1530	1778	0.84	1.03	1.09	0.98
3, 3, 4	1425	1300	1972	0.90	0.89	1.21	1.00
3, 4, 3	1720	1795	1805	1.08	1.21	1.11	1.13
3, 5, 2	1765	1400	1512	1.11	0.94	0.93	0.99
3, 6, 1	1520	1497	1367	0.96	1.01	0.84	0.93
4, 1, 5	1857	1612	1650	1.16	1.09	1.01	1.09
4, 2, 4	1475	2075	1775	0.93	1.39	1.09	1.13
4, 3, 3	1630	1435	1702	1.03	0.97	1.04	1.01
4, 4, 2	1712	1710	1527	1.08	1.15	0.94	1.06
4, 5, 1	1307	1500	1835	0.82	1.01	1.13	0.98
5, 1, 4	1952	1542	1627	1.23	1.02	1.00	1.08
5, 2, 3	1032	1645	2135	0.65	1.11	1.31	1.02
5, 3, 2	1713	1450	2263	1.07	0.98	1.39	1.14
5, 4, 1	1785	1740	2188	1.12	1.17	1.34	1.21
6, 1, 3	2020	1690	2182	1.27	1.14	1.34	1.25
6, 2, 2	1280	1258	2232	0.84	0.85	1.37	1.02
6, 3, 1	1853	1452	2180	1.16	0.98	1.34	1.16
7, 1, 2	1970	1635	2235	1.24	1.10	1.37	1.24
7, 2, 1	1342	1540	2250	0.84	1.02	1.38	1.05
8, 1, 1	1952	1637	2132	1.29	1.10	1.31	1.23

Average of 8 controls Series A, 1589; B, 1477; C, 1622.

on each plot were harvested, they were tied together in a bundle and allowed to dry. Later they were thrashed in a small machine by hand. These grain weights given in the table are not to be considered as absolutely correct since there are losses in the field by shattering, by birds, by transportation and by thrashing. These losses are comparatively small and perhaps uniform with all bundles.

The figures above the rectangles in fig. 9 represent the actual yields in grams, the first one to the left end of the first rectangle and the second to the right end of the second rectangle, and so on, arbitrarily arranged in this way to avoid crowding. The figures under the rectangles represent the normal yields calculated from the two nearest checks on the assumption that the fertility of the soil undergoes a gradual change from one check plot to the next. The yields of the fertilized plots between two checks were thus corrected. The yields of the fertilized plots beyond the checks to the end of the experimental strip were calculated in the same way, using the nearest check and the highest or lowest yielding plot as the case might be, when the fertility of the intervening space increased or decreased.

The actual yields and the relative weights of grain for the three series are given in table IX. In columns 5, 6, and 7 the relative yields are given in ratios, taking the values of the control as unity. In series A, all the checks averaged 1589 grams; in series B 1477 grams; and in series C, 1622 grams. In the last column, the average of all three series is given. The normal yields and relative weight of grain for series A, B, and C are given in table X. The ratios of fertilizer plots to check plots, with check as unity, are also given for the three series. The good and bad yields are represented on the triangular diagram as shown in fig. 11.

A study of the tables and figures reveals the fact that the plots in series C as a whole showed the highest yields. Series A followed next, and then series B. A study of each series showed that as far as high and low yields were concerned there was some similarity. When the data were arranged in the triangular diagram, the high yielding plots were for the most part found in the upper part of the triangle, and the low yielding cultures in the lower part of the triangle. In this respect, the data in Series A and C were more striking than in series B. When an average of the three series was taken, the best yielding cultures were with one exception in the upper angle above the 40 per cent. line. Furthermore, the yields were increasingly heavier as the cultures approach the upper angle. The poorest cultures are for the most part found in the lower part of the triangle in the 10 per cent. line. Consequently, it is apparent that plots high or moderately high in calcium acid phosphate give the highest yields while those low in acid phosphate give the lowest yields. The ill effects of too much K_2SO_4 in the fertilizer is apparent from a consideration of the tables. The cultures

TABLE X

NORMAL YIELD AND RELATIVE WEIGHTS OF OATS IN THE FIELD FROM MAY 8TH TO AUGUST 9TH. EXPERIMENT RUN IN TRIPLICATE, A, B, C

PLOT NUMBER	YIELD			YIELD RATIO TO CHECK AS UNITY			
	A	B	C	A	B	C	AVERAGE
	gm.	gm.	gm.				
1, 1, 8	1425	1220	1675	0.90	0.83	1.03	0.92
1, 2, 7	.....	1289	1460	.....	0.87	0.90	0.88
1, 3, 6	1325	1288	1370	0.83	0.87	0.84	0.85
1, 4, 5	1547	1288	1294	0.97	0.87	0.80	0.88
1, 5, 4	1794	1287	1308	1.12	0.87	0.81	0.93
1, 6, 3	1839	1386	1322	1.16	0.87	0.81	0.95
1, 7, 2	1636	1361	1336	1.03	0.92	0.82	0.92
1, 8, 1	1433	1437	1350	0.90	0.97	0.83	0.90
2, 1, 7	1410	1590	1364	0.89	1.10	0.84	0.94
2, 2, 6	2050	1400	1390	1.29	0.85	0.86	1.03
2, 3, 5	2100	1380	1422	1.32	0.93	0.88	1.04
2, 4, 4	1719	1360	1466	1.08	0.91	0.90	0.96
2, 5, 3	1528	1340	1488	0.96	0.91	0.92	0.93
2, 6, 2	1377	1320	1502	0.87	0.90	0.93	0.90
2, 7, 1	1417	1387	1504	0.89	0.94	0.93	0.92
3, 1, 6	1457	1348	1506	0.92	0.99	0.93	0.95
3, 2, 5	1497	1530	1508	0.94	1.03	0.93	0.96
3, 3, 4	1538	1300	1672	0.97	0.88	1.03	0.96
3, 4, 3	1720	1795	1805	1.08	1.21	1.11	1.13
3, 5, 2	1650	1673	1704	1.04	1.13	1.05	1.07
3, 6, 1	1600	1756	1602	1.00	1.18	0.99	1.05
4, 1, 5	1560	1839	1572	0.98	1.24	0.97	1.06
4, 2, 4	1569	2065	1644	0.98	1.39	1.01	1.12
4, 3, 3	1579	1888	1716	0.98	1.27	1.05	1.10
4, 4, 2	1588	1710	1788	1.00	1.15	1.10	1.09
4, 5, 1	1598	1500	1835	1.00	1.01	1.13	1.04
5, 1, 4	1952	1542	1627	1.23	1.04	1.00	1.09
5, 2, 3	1363	1645	2135	0.87	1.11	1.32	1.10
5, 3, 2	1713	1450	2263	1.08	0.99	1.39	1.15
5, 4, 1	2025	1503	1998	1.28	1.01	1.23	1.17
6, 1, 3	1987	1580	1981	1.25	1.07	1.22	1.18
6, 6, 2	1949	1603	1964	1.23	1.08	1.20	1.17
6, 3, 1	1911	1626	1947	1.20	1.10	1.17	1.15
7, 1, 2	1873	1635	2235	1.28	1.11	1.37	1.22
7, 2, 1	1874	1600	2250	1.18	1.08	1.38	1.21
8, 1, 1	1952	1637	2132	1.23	1.10	1.32	1.21

Average of eight checks A, 1589; B, 1477; C, 1622.

where high concentrations of this salt are found show lowest yields. The grouping, however, is not ideal except in series C. This is the state of affairs when actual yields are considered.

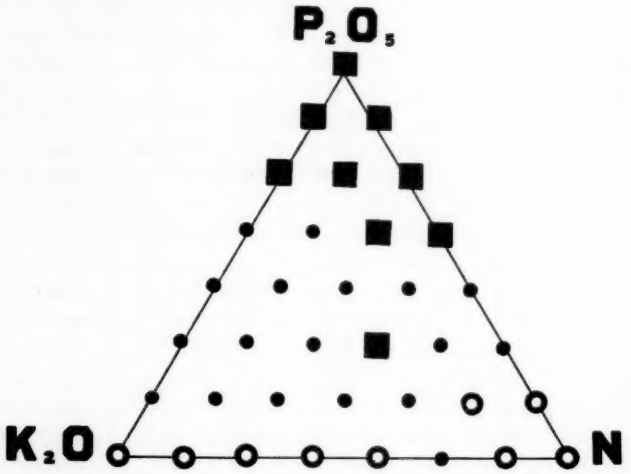

FIG. 11. High and low yielding cultures when *normal* yields are taken. The best cultures, ■ ; the poorest cultures, ○.

When normal yields were considered, better grouping of high yielding cultures was obtained with one exception, plot 3, 4, 3. Low yielding cultures are at the lower side of the triangle where acid phosphate is low in concentration.

For the particular soil and for the climatic and edaphic conditions under which the experiment was performed, the data showed that best growth was obtained where cultures were high in calcium acid phosphate and poor growth followed where low concentrations of calcium phosphate were used. This is in agreement with the general observation that cereals do best with comparatively high applications of acid phosphate.

The result of this experiment conducted in the field gave better proof of the need of a narrow range of ratios than the one conducted in the greenhouse. A single experiment like this, however, does not give one the needed proof that it is generally true, although it may look so. The experiment was not repeated the following year, the land having passed out of our hands. It is not known, therefore, whether the same results could have been obtained with repetition on the same field. Our chief concern was

to determine whether there was any need of a narrow range of ratios on a single soil type, during a single season.

Since the above experiment was completed, three other similar ones were conducted in different fields for the growing season of three successive years, 1919, 1920, and 1921. The high yields were not grouped together, nor were the low yields centered in any particular region. Thus, different types of soils have been tested during the four different seasons in an attempt to discover whether there is a definite grouping in response to salt ratios. Although tendencies indicating grouping were evident at times, one is not justified in concluding that for general practice any definite ratios are required. In fact, a rather wide range of ratios proved equally good. Increasing the application of active ingredients from 100 pounds to 160 pounds per acre, and running the experiment in duplicate did not change the general results. Decreasing the total amount of active ingredient brought no change in the general result. Three acres of corn on a farm near Clio, Michigan, yielded no better results. If one had carried on the experiment in the same field, year after year, the fertility might have been built up and the yields per plot greatly altered, but this is another type of experiment in itself, and one that was not attempted.

A more detailed account of the various experiments mentioned above is unnecessary. The results are of such a nature as to indicate that mineral salt ratios for good development of oats and other crops are not sharply defined in soil cultures. It seems improbable that such experiments will contribute much to the development of a rational fertilizer practice.

Summary

This paper presents the results of an experimental study of the theory of physiological balance in soil cultures. The method of attack is that suggested in 1910 for water culture investigations by SCHREINER and SKINNER and further developed by B. E. LIVINGSTON and his collaborators; in short it is the well known triangular system for determining the best salt ratio or combination for plant growth. The first section of the paper deals with the growth of Marquis wheat in soil in 2-gallon glazed pots. This experiment was conducted in the greenhouse during the period from December 16, 1918, to June 9, 1919. The plants were grown to maturity. The second section deals with the growth of Worthy oats in the field, during the period from May 8, 1918, to August 9, 1918. These plants also were grown to maturity.

In both experiments the soil was treated with all the possible combinations of the three ingredients in the fertilizer mixture when these were made arbitrarily to vary by increments of ten per cent. Consequently

there were thirty-six different ratios or combinations. The salts or fertilizer ingredients for both experiments were taken from the same supply and consisted of calcium acid phosphate, potassium sulphate, and sodium nitrate. The soil for the experiment reported in the first section of this paper was taken from the surface at different places scattered over the field, in which was conducted the experiment described in the second section.

In the greenhouse experiment each pot, and in the field experiment each row, represented a single fertilizer ratio or combination. Besides these ratios of the three salts, there were in both experiments single fertilizer applications for the purpose of comparison. In each experiment there were several control cultures.

The experiment in the field was run in triplicate. Each series, separated by alleys five feet in width, was made up of plots forty feet by two feet. Each plot contained four rows of plants, the two center rows receiving the fertilizer application broadcasted between them, when the plants had reached an average height of six to eight inches. In each series there were eight check rows scattered among the plots.

Certain observations were made on the environmental conditions surrounding the plants in both experiments. The importance of these observations is emphasized. The data collected were subjected to statistical analysis. This field experiment was preliminary in nature and the forerunner of three other series, one for each of the growing seasons of 1919, 1920, and 1921. The main conclusions of this study can be briefly stated.

The data, along with other unpublished data, suggest that nothing can be gained by attempting, through the use of the triangular system of fertilizer ratios, to find a definite ratio of salts, or in other words a physiological balance in soil cultures. The evidence does not indicate that we are justified in drawing any other conclusion than that for cereals, such as wheat, oats, and corn, the yields may be quite identical under somewhat wide variations of fertilizer ratios.

The many factors that were suggested as possible agents in producing the negative results were considered, but no satisfactory explanation has been found. It is suggested that the crops tested have been for generations so modified by domestication that they have become accustomed to wide variations in environmental conditions and for that reason are not suitable indicators for studies of this kind. Results might have been different with some wild variety raised in a restricted environment. It is quite apparent from knowledge already acquired that different proportions of the essential elements are required for the different phases of growth. These proportions probably vary also from week to week through the life of the plant, chiefly because there is a variation in the salt content of all ordinary soils. The

writer is convinced that a rather wide range of salt ratios can be used without causing appreciable differences in yields.

Under the conditions of these experiments the use of single fertilizer ingredients does not give as good results in soil cultures as a combination of three. Other experiments not only in soil cultures but in water cultures agree with and emphasize this conclusion. Only under conditions where the price for a mixture of the three fertilizer ingredients is prohibitive would the use of single fertilizers be advisable.

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ON THE PASSAGE OF BORIC ACID AND CERTAIN SALTS INTO FRUITS AND VEGETABLES

LOUIS KAHLENBERG AND RALPH TRAXLER

Introduction

In a previous paper[1] KAHLENBERG showed that boric acid passes through the living human skin by osmosis. This work suggested that boric acid might similarly pass into fruits and vegetables immersed in solutions of the acid. Experiments were not confined to boric acid alone, but a number of salts were tested to see if they would permeate the integuments of the same fruits and vegetables. The salts chosen for this purpose were Li_2SO_4, LiCl, $LiNO_3$, $Na_2B_4O_7$, $Li_2B_4O_7$, KI, $BaCl_2$, and $SrCl_2$. In a few instances still other salts were employed which will be mentioned in connection with the specific experiments to be described. The fruits and vegetables tested were cherries, strawberries, plums, gooseberries, grapes, tomatoes, cucumbers, carrots, peaches, and apples.

The method of experimentation was quite simple. It consisted of suspending the fruit in a solution of known strength of each of the substances mentioned, and then after a measured time determining whether any of the dissolved substance had entered the fruit. All of the substances used were chemically pure and they were dissolved in carefully distilled water. Usually the solution contained one-tenth of a gram molecule of the substance per liter, though in some cases saturated solutions were employed. The analysis of the fruit at the conclusion of the experiment was generally readily effected. Boric acid and borates could easily be detected by the turmeric test, and the spectroscope was used to detect lithium and strontium. Barium was detected by means of sulphuric acid, and iodine by the starch test. Before making the analysis the fruit was, of course, in each case very carefully washed with distilled water.

All of the fruits used were chosen with great care, and any fruits that showed imperfections were discarded. The experiments were conducted at least in triplicate in each case so as to guard against the possibility of error due to individual peculiarities of the fruit used. Moreover, the fruits employed were perfectly sound and had not been subjected to cold storage treatment except in a few cases where fruits which had been in cold storage were purposely employed to determine what effect such cold storage would have. In every case blank tests were made on the untreated fruit.

Before immersing fruits in the solutions, they were carefully washed

[1] KAHLENBERG, LOUIS, Jour. Biol. Chem. **62**: 149–156. 1924.

with distilled water and gently dried with a soft clean cloth. In a few instances the fruits were washed with alcohol, ether, chloroform, benzol, acetone, carbon bisulphide, etc., to remove the outer waxy coat before introducing the fruit into the solution. The results of these special experiments will be given later.

In immersing the various fruits special care was taken not to have either the stem ends or blossom ends dip into the liquids. All experiments were performed at room temperatures, about 20° C.

Results of experiments

CHERRIES.—The experiments with cherries were done with unripe sour cherries, with ripe sour cherries, and with ripe black California cherries. The unripe sour cherries were picked in the orchard of the Agricultural College of the University of Wisconsin at a time when the fruit was just beginning to turn white. The fruit was picked so that two cherries were connected by their stems. Each such pair was then hung over a small stick placed across the top of a crystallizing dish 10 cm. in diameter. Not over 30 minutes elapsed between the time of picking and of placing in the solution. The results are as follows: In 5.5 hours saturated H_3BO_3 solution, 0.1 molar LiCl, 0.1 molar $LiNO_3$, and 0.1 molar Li_2SO_4 passed into the cherries in detectable quantities. These amounts were greatly increased in 22 and 30 hours, respectively. A 0.1 molar solution of $Na_2B_4O_7$ passed into the cherries very slightly in 5.5 hours, but after 22 and 30 hours quite notable amounts were present in the fruit. It took 22 hours before 0.1 molar H_3BO_3 was found in the cherries in detectable quantity. Similarly, a saturated solution of $Li_2B_4O_7$ did not enter the fruit in notable amounts until after 22 hours. Even at that time, the tests for lithium and boron in the cherries were but slight. In 0.1 molar Li_2SO_4 they burst after 22 hours' immersion.

Similar experiments made with ripe sour cherries from the same trees yielded the following results: From a saturated solution, boric acid did not pass into the fruit until after it had been immersed for 21 hours. Similarly, it required 22 hours for lithium to make its appearance in the cherries from 0.1 molar solutions of LiCl, $LiNO_3$, and Li_2SO_4. From 0.1 molar H_3BO_3 it required 30 hours before the boric acid could be detected, while from 0.01 molar $Li_2B_4O_7$ but mere traces of lithium and boron were found in them after 53 hours. A 0.1 molar solution of $BaCl_2$ did not enter in detectable quantities until 52 hours had elapsed, while 0.1 molar $SrCl_2$ did not enter at all.

The leaves adjacent to the growing cherries were immersed in each of the solutions above mentioned in order to ascertain whether any of the dissolved

substances would thus pass into the fruit. These experiments were run from 5 to 70 hours, but in no case was even a trace of boric acid or the various salts found in the cherries.

California cherries were treated in the same manner as the sour cherries. The identical solutions already mentioned were used throughout. In addition, however, 0.1 molar solutions of each of the following were also employed: Sodium acetate, $CdBr_2$, NaF, KCl, NaCl, urea, Na_2SO_4, K_2SO_4, HCl, HNO_3, acetic acid, and distilled water. In most of these cases the cherries burst, the only exceptions being in the solutions of $BaCl_2$, $SrCl_2$, $CdBr_2$, and saturated solution of Na_2SO_4. In these latter cases no effect whatever was noted, though the experiments with $BaCl_2$ and $SrCl_2$ were continued for 54 hours and that with $CdBr_2$ for 150 hours. The saturated solution of Na_2SO_4 was used for 10 hours. All experiments were run at least in triplicate and continued till the cherries burst or clearly showed signs of decay. The bursting occurred at somewhat different times in the various solutions but generally in from 4 to 16 hours. The cherries were tested before they had actually burst, but in no case had any of the substances entered into them in detectable amounts before the bursting occurred.

The California cherries were bought at a fruit store in Madison. They had been shipped in refrigerator cars. The fruit was dark red, firm, and apparently in excellent condition. Immersed in distilled water, these cherries burst in from 4 to 5 hours. In the various solutions, the bursting did not occur until the fruit had been immersed somewhat longer, except that in 0.1 molar Na_2SO_4 the bursting occurred in 2 hours and in 0.05 molar H_2SO_4, HCl, and HNO_3 the bursting occurred in about 15 minutes.

Strawberries.—Fresh ripe strawberries were obtained from the beds of the Agricultural College. Due to unfavorable weather, the crop was small and for this reason but few experiments were made. Berries that had been shipped in were found to be unsuitable because they were all somewhat bruised.

Tiny hammocks of cheesecloth were suspended in the solutions, and the strawberries were placed in these hammocks in such a manner that the fruit was about half immersed in the solutions. The solutions consisted of saturated H_3BO_3, saturated $Li_2B_4O_7$, and 0.1 molar $Na_2B_4O_7$, H_3BO_3, $LiNO_3$, Li_2SO_4, and LiCl. In each of these cases the substances had passed into the strawberries in detectable amounts at the end of 5 hours. It was observed that the Li_2SO_4 passed into the strawberries in copious quantities as compared with the other substances used.

Plums.—The plums used in the experiments were: California blue "Diamond" plums, California yellow plums, and red "Miner" plums grown near Madison. The California plums were purchased at a local fruit store. They were in excellent condition and had probably been exposed to refrig-

eration in shipment. The blue plums were large, measuring 4 or 5 cm. in diameter, and the yellow ones were of about the same size. The blue and red plums were immersed in the following solutions, all experiments being done in triplicate at least: Saturated H_3BO_3, saturated $Li_2B_4O_7$, and 0.1 molar H_3BO_3, LiCl, Li_2SO_4, $LiNO_3$, $Na_2B_4O_7$, $SrCl_2$, and KI. The plums burst in 0.1 molar H_3BO_3 after 72 hours, in 0.1 molar $LiNO_3$ in 46 hours and 0.1 molar KCl in 44 hours. In saturated H_3BO_3 solution some of them burst in 36 hours. Li_2SO_4 penetrated the fruit more rapidly than any other salt. The other substances followed in the following order: $LiNO_3$, LiCl, $Li_2B_4O_7$, saturated H_3BO_3, $Na_2B_4O_7$, KCl, $BaCl_2$, $SrCl_2$. In fact only after 46 hours was it possible to detect H_3BO_3 in the plums from the saturated solution. No H_3BO_3 passed into the plums from the 0.1 molar solution, this experiment coming to an end in 72 hours when the plums burst. The $Na_2B_4O_7$ did not enter until 72 hours had elapsed. From the $Li_2B_4O_7$ solution some of the salt had passed into the fruit at the end of 27 hours, it being possible to get a spectroscopic test for lithium, but not a turmeric test for boron, which is not surprising since the latter test is less sensitive.

In no case did $SrCl_2$, $BaCl_2$ or KCl enter. In $SrCl_2$ the plums were immersed for 72 hours; in $BaCl_2$ for 96 hours; and in KCl for 44 hours, when the fruit burst.

Only a limited supply of yellow plums could be obtained, hence only a few experiments could be conducted with them. Here again, it was observed that neither $SrCl_2$ nor $BaCl_2$ entered the plums, though the experiments were run for 77 hours. After immersion in saturated H_3BO_3 solution for 48 hours, the boric acid had entered. In 0.1 molar H_3BO_3 the plums burst badly in 36 hours. The same occurred in 48 hours in 0.01 molar Li_2SO_4 and $LiNO_3$ entered the plums in notable amounts after 48 hours, while during that time $Na_2B_4O_7$ had entered in but mere traces.

The red plums had just been picked and had not been exposed to refrigeration. They were from 2 to 2.5 cm. in diameter, had red skins and a deep yellow pulp. The experiments clearly showed that these plums were much more permeable to the substances used than the yellow and blue California plums.

Immersed in 0.1 molar H_3BO_3 these plums burst in every case before any of the acid could be detected in them. The $SrCl_2$ again did not enter the plums even after 130 hours. A few of them burst in this solution at the end of 7 and 26 hours, though most of them did not burst. $BaCl_2$ was found in traces after 72 hours and in notable amounts after 144 hours. KI entered the fruit in notable amounts in 22 hours. Li_2SO_4 passed into the plums rapidly, notable quantities being present in 22 hours. However, in this solution some of them burst very badly, even at the end of 3 hours. From 0.1 molar solution H_3BO_3 did not enter. In one case the fruit burst

in 22 hours. A 0.1 molar solution of $Na_2B_4O_7$ entered the plums in notable quantities in 45 hours, but only an extremely faint test was obtained at the end of 22 hours. Saturated $Li_2B_4O_7$ entered in 22 hours in clearly detectable quantities. One of the plums burst in the solution at the end of that time. A 0.1 molar LiCl solution passed into the plum in 8 hours, although the test was quite faint; but in 22 hours notable amounts were found in them. Some of the fruit had burst at the end of that time. The $LiNO_3$ entered the plums a little more rapidly than the LiCl.

Experiments were made with the red Miner plums to see whether the fruit changed in weight while immersed in the solutions already mentioned. These experiments were commonly conducted for 4 hours, though in a few cases they were continued for 72 hours. As a rule the plums showed, if anything, a few hundredths of a gram diminution in weight; but the changes were so slight that they were almost within the limits of experimental error. It is consequently deemed unnecessary to recount all the weighings in tabular form here.

GOOSEBERRIES.—The gooseberries were large perfect fruits freshly picked from the garden. Not more than 30 minutes elapsed between the time of picking and their immersion in the solution. Here again neither $SrCl_2$ nor $BaCl_2$ entered the fruit though the experiments were conducted for 192 hours. KCl also found great difficulty in making its way into these berries, mere traces being found to have entered after 120 hours. $Na_2B_4O_7$ also did not pass into the berries for 144 hours, but at the end of 168 hours traces made their appearance in the fruit. H_3BO_3 did not enter from the saturated solution until after 120 hours. From 0.1 molar H_3BO_3 the acid entered in traces after 168 hours, but from 0.01 molar H_3BO_3 none of the acid entered the fruit. On the other hand, from 0.01 molar $Li_2B_4O_7$ lithium entered the berries in 96 hours and both boron and lithium were plainly detectable after 144 hours. 0.1 molar LiCl readily entered the fruit in 25 hours. One of the berries burst in this solution in 12 hours. The $LiNO_3$ did not make its appearance in the berries until after 48 hours. On the other hand, Li_2SO_4 entered in notable quantities in 48 hours. In this case Li_2SO_4 passed into the fruit more slowly than LiCl.

GRAPES.—Red California grapes, and red, white, and blue Wisconsin grapes were used in these experiments. The California grapes which had been shipped to Madison in refrigerator cars were purchased locally. Each grape was 1.5 cm. in diameter. The Wisconsin red and white grapes were obtained from the vineyards of the Agricultural College. The blue Wisconsin grapes were the so-called "Canton" grapes. They were grown in Madison. In each case from 1 to 2 cm. of the stem was left on the grape to be tested. By means of a thread attached to these stems the grapes were suspended in the various solutions.

In the case of the California grapes neither 0.1 molar $SrCl_2$, 0.1 molar $BaCl_2$, nor 0.1 molar H_3BO_3 entered the grapes, though the experiments were conducted for 72 hours, and in the case of the $SrCl_2$ for 96 hours. In the H_3BO_3 the experiments were continued for 144 hours. In a saturated H_3BO_3 solution it took 30 hours for H_3BO_3 to show its presence in detectable quantity in the fruit. The amount increased steadily up to 66 hours, when the experiment was discontinued. From 0.1 molar $Na_2B_4O_7$ the salt passed into them in detectable amount in 48 hours. Very material quantities of $Na_2B_4O_7$ were found in 144 hours. Immersed in saturated solution of $Li_2B_4O_7$, the grapes showed a faint spectroscopic test for lithium in 96 hours. This amount increased slightly up to 156 hours when the experiment was discontinued. However, even at this time not enough of the $Li_2B_4O_7$ had entered to yield the turmeric test for boron. It took 48 hours for lithium to pass into the grapes from 0.1 molar Li_2SO_4 solution. Curiously, even at the end of 96 hours the amount of lithium which had passed into the grape was exceedingly small, being only such traces as could be detected by the spectroscope. From 0.1 molar $LiNO_3$, lithium passed into the grapes in detectable amounts in 18 hours, and the quantity increased slightly but steadily up to 68 hours, the duration of the experiment. The grapes immersed in 0.1 molar LiCl exhibited spectroscopic traces of lithium after 24 hours, and the amounts increased notably in 66 hours. The KI entered the grapes from 0.1 molar solution in 18 hours, and the amount continued to increase up to 30 hours when the experiment was stopped.

Occasionally one of the grapes burst during the experiments, but these instances were so few and occurred so irregularly that they were no doubt due to individual differences.

Neither H_3BO_3, $Na_2B_4O_7$, $Li_2B_4O_7$, KI, $SrCl_2$, nor $BaCl_2$ entered the Wisconsin red grapes, although the experiments were prolonged to 196 hours and in some cases to 264 hours. From 0.1 molar Li_2SO_4 lithium was found in the grapes in 48 hours, and from 0.1 molar LiCl in 72 hours.

The white Wisconsin grapes were the Martha variety. They were found to be impermeable to all the solutions above mentioned, although the experiments were conducted for 120 hours and in some cases even over 300 hours. In fact the experiments were run up to the time when the grapes began to show decay.

Unripe grapes of the same Martha variety were similarly tested. The grapes were picked before any of the fruit on the vines had started to ripen. It was found that these unripe grapes were more permeable than the ripe ones. To be sure, in this case too, neither 0.1 molar $BaCl_2$ nor $SrCl_2$, $Na_2B_4O_7$, H_3BO_3 nor KI entered the grapes even after immersion for 144 hours. The boric acid also did not pass into the grapes from saturated solution in 144 hours. In some cases the experiments were conducted for

268 hours when the grapes began to show signs of deterioration. On the other hand, lithium entered these unripe grapes from 0.1 molar Li_2SO_4 and LiCl in 74 hours; from 0.1 molar $LiNO_3$, it passed into them in detectable quantity in 34 hours; and from 0.01 molar $Li_2B_4O_7$ in 144 hours.

The blue Wisconsin grapes known as the Canton grape were rather small, averaging about 1 cm. in diameter. They were carefully picked and at once tested in the laboratory. It was found that neither H_3BO_3, $Na_2B_4O_7$, LiCl, Li_2SO_4 nor KI entered the grapes, though the experiments were prolonged up to 240 hours.

The same Canton grapes were exposed to refrigeration, being kept in the ice compartment of a refrigerator for 76 hours. The fruit was placed in a box lined with cotton and did not come into direct contact with the ice. It was not frozen, although it had been chilled for 76 hours. The results of the osmotic experiments with these grapes were precisely the same as those with the grapes that had not been chilled.

Some of these Canton grapes were exposed to ultra-violet light from an Hanovia lamp for 2 hours, the grapes being placed about 16 inches from the lamp. The osmotic results with fruit which had been thus irradiated were the same as those with the untreated grapes.

TOMATOES.—Ripe tomatoes grown near Madison were carefully picked and at once immersed in the various solutions. After 6 hours, H_3BO_3 was found in the tomatoes which dipped into the saturated solution. It required 96 hours to get a faint turmeric test for H_3BO_3 in the tomatoes immersed in 0.1 molar H_3BO_3 solution; but in 72 hours 0.1 molar $Na_2B_4O_7$ entered them in detectable quantities. Lithium entered from saturated $Li_2B_4O_7$ solution in 36 hours, from 0.1 molar LiCl in 12 hours, from 0.1 molar Li_2SO_4 in 36 hours, and from 0.1 molar $LiNO_3$ in 6 hours. From 0.1 molar KI the salt entered in 6 hours. Barium was found in the tomatoes immersed in 0.1 molar $BaCl_2$ solution in 144 hours, but not a trace of strontium from 0.1 molar $SrCl_2$ even after 144 hours. Green tomatoes of the same variety were used. They varied from 6 to 8 cm. in diameter. Here again it was found that the green fruit offered less resistance to the solution than did the ripe fruit. In 6 hours H_3BO_3 passed into them from a saturated solution, though it required 36 hours to obtain even a faint test for boric acid in tomatoes immersed in 0.1 molar solution. A 0.1 molar solution of $Na_2B_4O_7$ entered the tomatoes in 36 hours. Saturated $Li_2B_4O_7$ penetrated them in 36 hours, in traces. Lithium passed into these green tomatoes from 0.1 molar Li_2SO_4 in 48 hours, from 0.1 molar $LiNO_3$ in 36 hours, from 0.1 molar LiCl in 36 hours. The 0.1 molar KI entered in 6 hours but neither $BaCl_2$ nor $SrCl_2$ passed into the green tomatoes in 120 hours, the duration of the experiment.

CUCUMBERS.—White spine cucumbers 7 to 10 cm. long and freshly picked were used in these experiments. All of the salts above mentioned entered

these cucumbers in notable quantities in from 16 to 20 hours except $SrCl_2$ which required 48 hours to make its appearance.

CARROTS.—The carrots, obtained from the College of Agriculture, were grown in soft, loose soil from which they were carefully removed without undue laceration of the rootlets. All of the substances mentioned entered the carrots in from 4 to 10 hours, except 0.1 molar H_3BO_3 which required 23 hours, 0.1 molar $SrCl_2$ 36 hours, and 0.1 molar $BaCl_2$ 24 hours. It was, of course, probable that the substances permeated the carrots where the rootlets were lacerated, it being quite impossible to remove the carrots from the soil without injuring the rootlets. Experiments were consequently performed later by watering the carrots with the various solutions while the plants were left growing in the soil. These results will be reported in another paper. Suffice it here to state that $BaCl_2$, $SrCl_2$, and KI did not enter the carrots in these experiments, and similar selective qualities were clearly exhibited toward the other substances.

PEACHES.—Perfect Elberta peaches purchased locally were used in these experiments. From saturated H_3BO_3 none of the solute entered the peaches till after 12 hours. It required 24 hours for H_3BO_3 to make its appearance in the peaches from 0.1 molar H_3BO_3, and 0.1 molar $Na_2B_4O_7$ entered in 44 hours, saturated $Li_2B_4O_7$ in 33 hours, 0.1 molar LiCl in 9 hours, 0.1 molar Li_2SO_4 in 24 hours, 0.1 molar $LiNO_3$ in 6 hours, 0.1 molar $BaCl_2$ in 24 hours, 0.1 molar KI in 12 hours. Again the $SrCl_2$ did not enter even after 120 hours.

It should be noted here that in some of the solutions in which the peaches were immersed, notably in the H_3BO_3 solutions, brown spots developed on the peaches which extended into the tissue of the fruit. These areas were soft and decayed, and contained H_3BO_3; but no H_3BO_3 was found in the clear area around the brown spots. Brown spots also appeared on the peaches immersed in $Li_2B_4O_7$, but LiCl and $LiNO_3$ caused no such spots to form, though these salts entered the fruit rapidly. On the other hand, Li_2SO_4 caused the skin of the peaches to decay badly. $BaCl_2$ caused brown spots to appear, and fair amounts of the salts were found in the uninjured part of the peach. KI solution did not discolor the peach skins, although iodine permeated them. In $Na_2B_4O_7$ solutions no brown spots were developed, though the solution became yellow after the peaches had been immersed in it for 40 hours. Finally in the $SrCl_2$, the peaches remained unchanged and this salt did not pass in even after 5 days, thus clearly demonstrating the highly selective action exhibited by the fruit.

CRABAPPLE.—The fruit used in these experiments was the Milton jelly crabapple. The apples were small, averaging 3 to 4 cm. in diameter. Salt did not pass into the crabapples from 0.1 molar solutions of $BaCl_2$, $SrCl_2$ or KI in 192 hours. Boric acid from saturated solution entered the fruit in

96 hours, and the apples immersed in 0.1 molar $Na_2B_4O_7$ solution showed detectable quantities of boron in 120 hours. Lithium from saturated solution of $Li_2B_4O_7$ was detected in the fruit in 48 hours. The 0.1 molar Li_2SO_4 entered the crabapples in 24 hours, 0.1 molar $LiNO_3$ in 24 hours, and 0.1 molar LiCl in 48 hours.

Further experiments were performed with the Milton jelly crabapples using the solutions above mentioned, but allowing the blossom end of the fruit to come into contact with the various solutions. Here H_3BO_3 from saturated solution entered the apples in 102 hours, from 0.1 molar solution in small amounts in 174 hours, and from 0.01 molar solution not at all in 174 hours, the duration of the experiment. Lithium entered from saturated $Li_2B_4O_7$ solution in spectroscopic amounts in 78 hours. The fruit was permeable to 0.1 molar $Na_2B_4O_7$ in 78 hours, 0.1 molar $LiNO_3$ in 28 hours; and to 0.1 molar Li_2SO_4 in 28 hours. In 0.1 molar concentration $BaCl_2$, $SrCl_2$ and KI did not pass into the fruit in 192 hours, the duration of the experiments. Very little difference in the rate of entrance of most salts was found in the case of the crabapples when only the sides dipped into the solutions as compared with the tests when the blossom end was immersed in the liquid. In a few cases the substances passed into the blossom end more rapidly than through the sides, showing that in experiments such as these it is best to prevent the solutions from coming into contact with any portion of the skin except the smooth and unbroken side of the fruit.

Apples.—Wealthy, Talent, Ben Davis, Black Ben Davis and Baldwin apples were used in these experiments. They were all picked in the orchard of the Agricultural College except the Baldwins, which were shipped from Michigan, but had never been in cold storage. In every case, care was taken that the solution did not come in contact with either the stem or blossom end of the fruit. Occasionally an apple was found that developed a decayed spot within a few hours after immersion in the liquid. Apples showing this effect always contained detectable quantities of the substance in the solution. The only explanation of this behavior is that the skins of these particular apples contained minute abrasions, probably caused by handling or shipping, which escaped detection when the fruit was sorted and selected for experimentation. The decayed areas indicated the spot through which the solution found an easy entrance into the apple.

The Wealthy apples were used for experimentation soon after picking from the trees. The fruit averaged 5 to 6 cm. in diameter. Boric acid from saturated solution, and from 0.1 molar solution did not appear in the Wealthy apples even after 400 hours of immersion. Potassium iodide from 0.1 molar solution did not enter in detectable quantities in 240 hours, the duration of the experiment. Although the apples were left in contact with saturated $Li_2B_4O_7$ solution for 240 hours no spectroscopic test for lithium

was found. Sodium biborate from 0.1 molar solution did not enter the apples even after 288 hours, neither did 0.1 molar $BaCl_2$ nor $SrCl_2$ enter after 400 hours. But 0.1 molar LiCl entered in 130 hours, 0.1 molar $LiNO_3$ in 252 hours, and 0.1 molar Li_2SO_4 in 252 hours.

By peeling one side of Wealthy apples and placing the peeled side in the solution it was found that 0.1 molar $BaCl_2$ passes through the tissue of the apple very readily and copiously, and the 0.1 molar $SrCl_2$ appreciably, although these substances do not pass through the apple skins in 400 hours.

Wealthy apples were quickly washed with ether, then with benzene, followed by ethyl alcohol. This treatment removed most if not all of the wax from the skins of the apples, but the washed apples turned brown within a few hours. It was found that benzene was the solvent which caused the greatest injury to the fruit. The washed apples were placed in the above mentioned solutions, and all of the substances entered in notable amounts in 26 hours except the $BaCl_2$, which required 96 hours, and the $SrCl_2$ which did not enter in 150 hours. This behavior of the $SrCl_2$ showed that the skin was not destroyed, nor were gross openings made during the washings, although the permeability to H_3BO_3 and various salts had been greatly increased.

The action of the vapors of various organic solvents on the skins of Wealthy apples was determined. A pipe stem triangle bent so as to form a tripod was placed in a large beaker, and the apple was put upon this tripod. The liquid to be tested was then carefully poured down the side of the beaker till the bottom of the latter was covered about 1 cm. deep. The beaker was then covered with a large convex glass and allowed to stand at room temperature, about 20° C. The experiment with each apple was usually continued for 24 hours. Benzene, chloroform, ether, carbon tetrachloride, carbon bisulphide, toluene, and acetone were used, each separately, as described. Acetone vapors were the only ones which did not cause marked visible injury to the apple skins. The vapors of the other six solvents caused the skins to be discolored and disintegrated so that when the apples were subsequently immersed in the aqueous solutions the latter readily penetrated the fruit. In an experiment to be mentioned later, apples were treated with boiling hot organic vapors in which case the effect just mentioned was greatly increased.

Wealthy apples were exposed to ultra-violet light from an Hanovia lamp for 2.5 hours. The temperature did not rise above 30° C. During the operation of the lamp a strong odor of ozone could be detected. During and after this treatment the natural odor of the apples was much enhanced.

These apples were found to be more permeable to 0.1 molar $Na_2B_4O_7$, saturated $Li_2B_4O_7$, 0.1 molar LiCl, $LiNO_3$ and Li_2SO_4 than were the untreated apples. The time required for $Na_2B_4O_7$ to enter decreased from 288

for the untreated, to 216 hours for the irradiated apples, for LiCl from 95 to 56 hours, for $LiNO_3$ from 250 to 44 hours, and for Li_2SO_4 from 252 to 90 hours. The irradiation did not affect the permeability of the skins toward H_3BO_3, KI, or $BaCl_2$, which fact seems specially noteworthy.

The Talent apples, 7 to 8 cm. in diameter, have skins which are green, with a russet coating in many instances. The substances used passed into the Talent apples much more copiously than into the Wealthy apples. Even 0.1 molar $BaCl_2$ entered the Talent apples in small amounts in 115 hours. It was found that the Talent apples with russet skins were much more readily permeated than those from the same trees but with clear green skins. Notable amounts of KI and H_3BO_3 were found in russeted apples in 90 hours, whereas no KI or H_3BO_3 was found in the clear green apples until after 115 hours and then only in small amounts. Of the lithium salts, 0.1 molar $LiNO_3$ entered the clear green apples in 19 hours, 0.1 molar LiCl in 30 hours, and 0.1 molar Li_2SO_4 in 43 hours. The 0.1 molar $Na_2B_4O_7$ entered russet apples in 115 hours, but not the clear skinned apples in 165 hours. The cause of russeting does not seem to be known. It is known, however, that russet apples lose moisture more readily than apples of the same variety without russet.

Talent apples were irradiated with ultra-violet light from the Hanovia lamp used in the previous experiments. In this particular case, however, very little ozone was produced in the neighborhood of the lamp. The irradiated apples showed no increased permeability to any of the solutions used, except in the case of 0.1 molar $Na_2B_4O_7$, which appeared to enter a little more rapidly and copiously than into apples not irradiated.

The Ben Davis apples used were obtained from the orchard of the Agricultural College. The fruit averaged 5 to 6 cm. in diameter. The skins of the Ben Davis apples were more permeable than those of the Wealthy apples and less permeable than the Talent apples to H_3BO_3 and KI. The H_3BO_3 from saturated solution entered in 168 hours and the KI from 0.1 molar solution in 288 hours. Lithium from 0.1 molar LiCl entered the Ben Davis apples in 130 hours. More experiments were not performed because of the small quantity of fruit available.

The Black Ben Davis apples were also 5 to 6 cm. in diameter and were exceptionally firm, which eliminated much of the trouble usually occasioned by bruising. Boric acid from saturated solution entered the Black Ben Davis apples in 140 hours, and 0.1 molar $Na_2B_4O_7$ passed into the fruit in 180 hours. Lithium entered the apples from 0.1 molar LiCl in 24 hours, from 0.1 molar $LiNO_3$ in 24 hours, and from 0.1 molar Li_2SO_4 in 18 hours. The apples immersed in saturated $Li_2B_4O_7$ contained spectroscopic amounts of lithium in 22 hours. Neither 0.1 molar KI nor $BaCl_2$ passed into the fruit in 300 hours, while no $SrCl_2$ could be detected in the tissues of the apples even after 55 hours of immersion.

Lithium malate was prepared from pure Li_2CO_3 and pure malic acid, and Black Ben Davis apples were immersed in a 0.1 molar solution of this salt. Lithium was detected in the apples after 19 hours had elapsed. The salt had passed into the fruit in copious amounts after 22 hours. The lithium thus entered the apples from the lithium malate slightly more rapidly than from LiCl and $LiNO_3$ and about as readily as from Li_2SO_4.

To test the influence of high temperatures on permeability, Black Ben Davis apples were exposed to a temperature of 75° C. for one hour in a copper oven with water jacketed walls. Thirty apples weighing approximately 3 kilograms were placed in the oven. While the apples were being heated the wax on the skins became soft and felt greasy when touched with the fingers, and the characteristic odor of baking apples was noticed. After an hour's heating the apples were allowed to come to room temperature, after which they were placed in the various solutions. The color of the fruit was changed from a beautiful red to a reddish-brown. Temperatures higher than 75° C. were tried, but found to cause ruptures in the skins of the apples. The permeability of the skins of these heated apples to the above mentioned substances was found to be almost the same as that of the fresh, raw apples. In a few cases it seemed that the permeability had slightly increased by the heating but the differences were so slight that they may very properly be attributed to individual differences.

Low temperatures were also used on Black Ben Davis apples, which were exposed to a temperature of −8° C. for 5 hours. The frozen apples were allowed to thaw out and come to a temperature of 20° C. when they were placed in the solutions used in the above experiments. In this case H_3BO_3 from saturated solution entered the apples in 50 hours and from 0.1 molar H_3BO_3 solution in 168 hours. Lithium was detected in the fruit immersed in 0.1 molar LiCl, $LiNO_3$ and Li_2SO_4 after 22 hours, also from saturated $Li_2B_4O_7$ after 22 hours. No KI was detected in the apples that had been frozen even after 118 hours and $BaCl_2$ was not found though the experiment ran for 168 hours.

Freezing the apples made it possible for most of the various substances to pass through the skins more readily and copiously than through the normal skins of the same variety of apples. The frozen apples immersed in the various solutions did not show the development of any decayed areas while in the liquid. This would indicate that no gross breaks or ruptures were made in the skins by the process of freezing, for such breaks are uniformly marked by a decaying of the fruit when in contact with the solutions.

Apples of the same variety were exposed to the vapors of boiling acetone for 20 to 30 minutes. The acetone removed most of the wax from the skins and also much of the coloring matter. In other experiments it was found that acetone was the only common solvent which removed the coloring matter, although all removed most of the wax from the skins.

The apples were allowed to cool and were then placed in the various solutions. After the treatment, H_3BO_3 entered from saturated solution in 26 hours very copiously, and $Na_2B_4O_7$ from 0.1 molar solution also entered very abundantly in 26 hours. Lithium entered the acetone treated apples from saturated $Li_2B_4O_7$ in 10 hours, and from 0.1 molar LiCl, $LiNO_3$ and Li_2SO_4 in 10 hours in very notable quantities. At the end of 26 hours, KI was found in the apples in large amounts and even $BaCl_2$ had entered by this time in notable quantities.

The boiling point of acetone is 56–57° C. It is not this temperature which caused the increased permeability, for the same variety of apples were exposed to a temperature of 75° C. without marked increase in the permeability of their skins. It consequently seems probable that the substances enter the apples more readily because the solvent has removed the waxy coating.

The Baldwin apples used for experimentation were obtained from a dealer who had obtained them from northern Michigan. The apples had never been in cold storage, but were wrapped individually in tissue paper and stored in a cool place in the basement of the chemistry building, from whence they were taken to the laboratory as needed.

In the Baldwin apples, H_3BO_3 from saturated solution could not be detected after 144 hours had elapsed, nor from 0.1 molar solution after 267 hours. From 0.1 molar solution, $Na_2B_4O_7$ was found in the apples after 146 hours. Lithium had passed into the apples from 0.1 molar LiCl in 80 hours and from 0.1 molar Li_2SO_4 in very small amounts in 50 hours; but 0.1 molar KI did not pass through the skins in 100 hours, the duration of the experiment. Barium chloride from 0.1 molar solution did not enter the Baldwin apples, although the experiment was continued for 312 hours. These apples were also immersed in 0.1 molar KCNS, but no trace of this salt could be found under the skin of the apple even after 122 hours of immersion. The skins of these apples were in general more permeable to the substances used than the Wealthy and Ben Davis apples, and less permeable than the skins of the Talent and Black Ben Davis apples. Each variety of apple, however, displays some peculiarities toward various individual reagents.

Baldwin apples were placed 18 inches from a mercury vapor lamp and were irradiated continuously for 2 hours. The odor of ozone was quite strong in the neighborhood of the lamp and the natural odor of the apples was much enhanced during and for a short time following the irradiation. After treatment, H_3BO_3 passed into the irradiated apples from saturated solution in 80 hours and from 0.1 molar solution in 267 hours, while from 0.1 molar solution $Na_2B_4O_7$ entered in 80 hours. Lithium was detected in the apples from 0.1 molar LiCl in 22 hours and from 0.1 molar Li_2SO_4 in

34 hours, and 0.1 molar KI entered the irradiated fruit in 80 hours. In every solution used, the solute passed through the skins of the apples which had been exposed to ultra-violet light for 2 hours much more rapidly and copiously than through the skins of the untreated fruit. These results are quite different from those obtained with the irradiated Wealthy apples.

To determine what effects ozone itself might produce, Baldwin apples from the same lot used for the above experiments were exposed for 20 minutes to an atmosphere of oxygen and ozone containing 2.25 grams of ozone per cubic meter of mixture. The ozone was produced by the action of the silent electric discharge on dry oxygen. After a short exposure to ozone, the natural odor of the apples was found to be much enhanced. For a few minutes after the apples were removed from the atmosphere of ozone, their waxy coating was very soft and sticky, and the subsequent hardening of the wax took place gradually. The apples were not placed in the solution until after the wax had hardened. Within a few hours after the treatment with the ozone-oxygen mixture, brown spots appeared at the lenticels of the apple skins. These small openings or pores could be seen with the naked eye in the normal fruit and it was evident that they were the locations at which the ozone took hold most strongly. It was shown in the case of the KI solution that it was only at these points or areas that the solution entered. Iodine could be detected in the tissue immediately under the spots, but at no other place in the tissue.

Boric acid from saturated solution entered the Baldwin apples treated with ozone in 24 hours and from 0.1 molar solution in 90 hours; lithium passed into the fruit from 0.1 molar LiCl in 10 hours and from 0.1 molar Li_2SO_4 in 20 hours; and KI was found under the affected spots after 42 hours. From 0.1 molar solution, $BaCl_2$ did not enter the apples although the experiment was continued for 312 hours. These results show that a very marked increase in the rate and extent of entrance of the various solutions used is caused by the action of ozone on the skins of the apples.

Finally, the effects of peroxide of hydrogen were studied. Baldwin apples were treated with H_2O_2 prepared by passing ozone into water and also by the action of H_2SO_4 on an excess of BaO_2. The concentration of the H_2O_2 ranged from 0.1 per cent. to 3.0 per cent. by weight. The apples were immersed in these solutions for 30 minutes and then placed in the various solutions, but no increased permeability was noted due to the action of H_2O_2. By using more dilute ozone the effect which that substance produces is naturally greatly lessened. It is still a question whether the increased permeability of the skins of irradiated apples is due to ozone formed by the ultra-violet rays.

The remarkable fact was observed that apples treated with H_2O_2 were much less permeable to Li_2SO_4 than the untreated apples, requiring 192

hours before lithium could be detected. The effect of the ozone on the apple skins was evidently due to some action of its own, therefore, and not to that of H_2O_2 formed by its reaction with the moisture present on the surface of the fruit.

Summary

1. Various fruits and vegetables were immersed in solutions, and the rapidity and copiousness with which the substances passed through the outer membranes determined. The solutions used were 0.1 molar and in a few cases saturated solutions of LiCl, $LiNO_3$, Li_2SO_4, $Li_2B_4O_7$, H_3BO_3, $Na_2B_4O_7$, KI, $BaCl_2$ and $SrCl_2$. Other solutions were used in a few experiments.

2. The skins on unripe sour cherries were found to be more permeable than the skins of ripe fruit from the same tree. Substances did not pass through the leaves and into adjacent cherries when the leaves were immersed in the solutions. California cherries burst before any of the substances in the solutions could be detected in the fruit.

3. Red Wisconsin plums were more permeable than either blue or yellow California plums. The permeabilities of the blue and yellow plums were about the same. The three varieties frequently burst during immersion, but without increase of weight.

4. LiCl, Li_2SO_4 and $LiNO_3$ were the only substances which passed through the skins of freshly picked gooseberries readily and copiously.

5. California grapes were found to be more permeable than Wisconsin grapes. The latter were found to be practically impermeable to all of the substances used except LiCl, $LiNO_3$ and Li_2SO_4, which passed through after long immersion. Irradiation with ultra-violet light did not cause increased permeability of the skins of the grapes.

6. Toward LiCl, $LiNO_3$ and Li_2SO_4 the skins of green tomatoes were less permeable than the skins of ripe tomatoes. The reverse was true for H_3BO_3, $Na_2B_4O_7$ and $Li_2B_4O_7$. The skins of the tomatoes were the only membranes investigated which were more readily permeated by H_3BO_3 and KI than by LiCl, $LiNO_3$, and Li_2SO_4.

7. All the substances used entered cucumbers and carrots quite readily and copiously, in the latter case probably because of the formation of openings through the laceration of the rootlets when the carrots were removed from the soil.

8. The only substance used which did not readily pass through the skins of Elberta peaches was $SrCl_2$. Some of the solutions used caused discoloration of the skin and tissues of the peaches.

9. Milton jelly crabapples, and Wealthy, Talent, Ben Davis, Black Ben Davis and Baldwin apples were found to show selective action toward the

various solutions used. Russet apples were found to be more permeable than the same variety without russet.

Irradiation with ultra-violet light and exposure to dilute ozone was found to cause increased permeability of the skins of apples to most of the substances tested, but H_2O_2 had practically no effect on the permeability of the membranes. Removal of the waxy coating from the skins caused a very marked increase in the rate and extent of entrance of substances into the fruit.

Freezing without rupturing the skins was followed by a more rapid and copious entrance of the substances, while on the other hand, heating to 75° C. had very little effect on the permeabilities of the membranes.

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NITROGENOUS METABOLISM OF *PYRUS MALUS* L.

I. INFLUENCE OF TEMPERATURE OF DESICCATION ON WATER-SOLUBLE NITROGENOUS CONSTITUENTS AND SEPARATION OF WATER-SOLUBLE PROTEIN FROM NON-PROTEIN CONSTITUENTS[1]

WALTER THOMAS

Introduction

Notwithstanding the fact that nitrogen occupies a unique position in plant metabolism, our knowledge concerning the processes of absorption, synthesis or ultimate disposition of its compounds in the metabolic cycle of the higher plants is very limited.

The various groups of the complex combinations which nitrogen is capable of forming may have quite different physiological functions and, accordingly, as SPOEHR and MCGEE (**23**) have already emphasized, it is improbable that a rational conception of the function of nitrogen in the higher plants can ever be obtained without further studies of the physiological rôle of its various groups. Thus, a knowledge of the part these groups play and the extent to which their reactivity can be controlled are questions that must be solved before the problems of growth, reproduction and movement can be solved.

Owing to the very large number and complexity of the compounds which nitrogen forms, the separation and identification of its various groups or compounds are beset with difficulties; but the advances made in the chemistry of proteins have paved the way for an approach to investigations on nitrogen metabolism that were not available to the older workers in this field (**9**).

The possibilities which lie in the acquisition of greater knowledge of these weighty problems by the more modern method of approach are indicated by Chibnall's (**2**) studies on the distribution of nitrogen in the leaves of the Runner Bean (*Phaseolus vulgaris*) throughout a vegetative cycle;

[1] Published by permission of the Director of the Agricultural Experiment Station as scientific paper no. 418 and contribution no. 24 from the Department of Agricultural and Biological Chemistry.

The cost of these investigations, forming a part of a cooperative project with the Department of Horticulture, has largely been defrayed by a grant from the Adams Fund for Agricultural Research.

but no investigations have been reported on the distribution or partition of nitrogen in the tissues of fruit trees throughout a cycle.

The present studies were undertaken for the purpose of obtaining data that might give an insight into the transformations taking place, as measured by the nitrogen fractions soluble in water, at the different vegetative periods throughout a year's cycle. When the possibilities and limitations of such studies are known it should then be possible to determine to what extent such information might be utilized, more especially in relation to the problems of growth and reproduction. The problem necessarily involves the simultaneous study of the validity of methods.

A. The effect of temperature of desiccation on the water-soluble nitrogenous constituents

As the nature of the problem necessitated the accumulation of samples for subsequent analyses, prevention of enzymatic activity as soon as possible after the samples are collected is essential. Preservation by means of alcohol is undesirable because of its precipitating effect on the proteins. Freezing also produces marked changes in some plants (**27**). The available evidence indicates that inactivation of proteolysis by dehydration by drying probably causes less changes in the tissues than any of the methods at present in use. The question of the optimum temperature at which autolytic changes are reduced to a minimum has received attention recently by Tottingham, Schulz and Lepkovsky (**27**) and by Link and Schulz (**17**). These papers and also a paper by Chibnall (**3**) contain a discussion (with references) of the temperatures adopted by previous investigators for the desiccation of the tissues of various plants. None of the earlier workers appear to have made any investigation into the effects of temperature on the composition of the plants under experiment.

Although Chibnall (**3**) found, in the case of the leaves of the Runner Bean, that low-temperature drying in a closed oven at 40–50° C. entailed protein autolysis, causing an increase in ammonium salts, asparagine and amino acids, the American investigators (**17, 27**), under the conditions adopted by them, found that the greatest changes produced either by rapid drying or freezing resulted from the coagulation of soluble proteins. Coagulation was greatest at high temperatures; at low temperatures (32–40° C.) both coagulation and proteolytic changes seemed to occur.

Experimental

The material used for this investigation was obtained from Stayman Winesap apple trees 15 years old growing in the College orchard. Samples of leaves and new shoot growth were collected in the early part of a July morning when active growth was in progress.

Temperatures adopted in this investigation

The experiences of previous investigators (**17, 27**) that very high and very low temperatures are to be avoided, minimum changes occurring at "intermediate" temperatures (50–70° C.), afford a sufficient basis for the selection of the three temperatures, 50, 60 and 70° C., for the determination of the optimum temperature causing minimum proteolytic changes in the water-soluble nitrogenous constituents of the species under investigation.

At 50 and 60° C. desiccation was carried out in ordinary Freas ventilated ovens. Drying at 70° C. was accomplished in wire bottom trays in a drying closet heated by steam coils. To further restrict the possibility of enzymatic activity the moisture content was reduced to 0.5 per cent. by transference to a vacuum oven for eight hours after the samples had been ground. The samples were preserved in glass stoppered sealed bottles. A month elapsed before analytical work was commenced on the dried samples.

Preparation of samples for analysis

The removal of the vacuole and colloidal cytoplasmic cell contents from the fresh leaves was readily accomplished by means of a Nixtamal mill (**19**) which readily disintegrates the cells of succulent plant materials after two or three passages through the mill. However, the disintegration of the cells of the refractory woody shoot growth could not be accomplished by this means. This was finally effected by the laborious process of alternate passage through an Enterprise mill and mincing by hand and afterwards macerating with quartz sand in the manner to be described later. The dried material was easily ground by a method previously described (**26**).

Method of extraction of the soluble nitrogenous constituents: Direct method versus Chibnall's procedure

Tottingham, Schulz and Lepkovsky (loc. cit.) found the direct extraction with water to be a satisfactory method for partitioning the products of the nitrogenous constituents of barberry and sugar beet leaves. Water enabled them to extract the greater part of the proteins exposed by crushing the cells of the fresh tissues, the direct method compared with Chibnall's (**4, 5**) giving somewhat higher values for total water-soluble nitrogen.

Comparison of the direct method with Chibnall's method by the writer on apple leaves indicated that only two-thirds as much protein N and non-protein N were found in the extracts obtained by the latter method. These results would indicate that the modifying action of the cytolytic agent (ether) in Chibnall's procedure is of greater influence than the post-mortem changes due to the "setting" of the cytoplasm into a gel (**6**).

All extractions were made by grinding 50 to 100 gm. of the material in a mortar with water (about 200–300 cc.) containing 20 cc. of 2 per cent. phenol. The brownish red extract was decanted on a filter of paper pulp, using a Buchner funnel, and the residue, after washing with water, again transferred to the mortar for further grinding. The process of filtering and grinding was repeated until microscopic examination showed that 85–90 per cent. of the cells were broken up. All samples were made up to a volume of 2,200 cc. and covered with a layer of toluene.

Methods of analysis

The methods of determining the various fractions will be given in detail in the third paper of this series in connection with the partition experiments.

TABLE I

Total water-soluble N, non-protein N, ammonia N and amide N in the leaves

a. As percentages of the dry matter

Series number	Total N	Total water soluble N	Non-protein N	Ammonia N	Amide N
OA	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Fresh	1.80	0.130	0.089	None	0.014
Dried at 50°	1.82	0.120	0.090	None	0.016
Dried at 60°	1.82	0.120	0.092	.....	.....
Dried at 70°	1.83	0.109	0.098	None	0.016
18					
Fresh	1.86	0.146	0.120	0.003	0.013
Dried at 50°	1.86	0.144	0.124	0.006	0.017
Dried at 60°	1.85	0.144	0.123	0.006	0.017

b. As percentages of the total nitrogen of the dry matter

Series number	Total N	Total water soluble N	Non-protein N	Ammonia N	Amide N
OA	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Fresh	1.80	7.22	4.94	.....	0.77
Dried at 50°	1.82	6.59	4.94	.....	0.88
Dried at 60°	1.82	6.59	5.05	.....	.....
Dried at 70°	1.83	5.95	5.35	.....	0.87
18					
Fresh	1.86	7.85	6.45	0.16	0.70
Dried at 50°	1.86	7.74	6.66	0.32	0.91
Dried at 60°	1.85	7.59	6.65	0.32	0.91

In the present experiments the effects of desiccation on the sap contents was followed by observation of the changes in the total water-soluble N, non-protein N, ammonia N and amide N.

The results obtained are given in tables I and II.

TABLE II

TOTAL WATER-SOLUBLE N, NON-PROTEIN N, AMMONIA N AND AMIDE N IN THE NEW GROWTH

a. AS PERCENTAGES OF THE DRY MATTER

SERIES NUMBER	TOTAL N	TOTAL WATER SOLUBLE N	NON-PROTEIN N	AMMONIA N	AMIDE N
OB	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Fresh	0.715	0.163	0.099	0.003	0.009
Dried at 50°	0.715	0.155	0.110	0.004	0.014
Dried at 60°	0.711	0.155	0.104	.....	.....
Dried at 70°	0.710	0.137	0.103	0.004	0.011

b. AS PERCENTAGES OF THE TOTAL NITROGEN OF THE DRY MATTER

SERIES NUMBER	TOTAL N	TOTAL WATER SOLUBLE N	NON-PROTEIN N	AMMONIA N	AMIDE N
OB	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Fresh	0.715	22.8	13.8	0.419	1.250
Dried at 50°	0.715	21.6	15.3	0.559	1.950
Dried at 60°	0.711	21.8	14.6	.....	.....
Dried at 70°	0.710	19.3	14.5	0.563	1.549

Discussion

These results, as also those of TOTTINGHAM and his co-workers (**27**), indicate that the higher the temperature (within limits) the greater becomes its influence on the coagulation of soluble proteins. In the case of the woody branch growth this appears to be accompanied by greater proteolytic changes at the lowest temperature (50° C.), a possible explanation of which is the longer time required for the desiccation of ligneous tissues.

It is apparent, however, that desiccation of the tissues of this plant species, which is low in water-soluble nitrogen, under the temperature conditions described, does not greatly change the proportions of total water-soluble N and non-protein N and that the changes in the ammonia N and amide N are not, except in the woody tissues, much greater than the error of determination. The optimum temperature appears to be around 60° C.

These findings and those of LINK and SCHULZ (17) on the barberry suggest that the proteolytic enzymes, due possibly to the different nature of the nitrogenous or carbohydrate constituents, may be relatively less active in plants low in water-soluble nitrogen than in those that are relatively high in these constituents. But whether, as suggested by LINK and SCHULZ, the differences observed between the high and low water-soluble nitrogen types of plants are due to the initial water content and the rate of its removal can, it seems to the writer, only be determined by further investigations in which plants low in the soluble cytoplasmic proteins are compared with those that are high (7). Until such questions are cleared up each plant tissue must represent a separate problem, in which case generalizations extended to all types of tissues are impossible.

B. THE SEPARATION OF THE PROTEINS FROM THE NON-PROTEIN CONSTITUENTS

Although, for the accurate determination of the amino nitrogen content, the separation of the proteins from the other nitrogenous constituents of the extracts of plant cells is by some investigators (24) considered unnecessary, others (28) have shown the advantage of doing so. The water-soluble protein in the plant species under investigation is, as we have seen, quite low; nevertheless, apart from the desirability of having some knowledge concerning its amount and fluctuations during the vegetative cycle, positive evidence was obtained that the removal of the soluble protein by the method to be described herein enabled more consistent results to be obtained in the estimation of the several non-protein fractions with which these metabolism investigations are mainly concerned.

The objective is to effect the separation with the removal of as little as possible of the intermediate products of protein degradation; but whether any reagent accomplishes the complete exclusion of all products which are only a little below the original proteins in their complexity from the protein-separates is doubtful.

NOTES ON THE REAGENTS USED IN THIS INVESTIGATION

Since STUTZER (25) proposed copper hydroxide for the removal of "peptides" from the "amide N," a number of reagents, such as methyl alcohol, acetic acid, trichloroacetic acid, phosphotungstic acid, tannic acid, and colloidal ferric hydroxide, have been used for the separation of the "protein" from the "non-protein" constituents, and, unfortunately, the evidence points to the conclusion that a reagent satisfactory for one kind of biological material may be unsuitable for another.

HART and BENTLEY (13) have shown that STUTZER's reagent may not, in all cases, effect a complete separation of all protein and polypeptide

structures from amines and amides, for, although the copper salts of a number of di-, tri-, and tetra-peptides have been prepared, having the formula $(peptide)_1$:Cu (**16**), not all peptides may form insoluble copper salts under the conditions usually adopted for the precipitation. From the standpoint of the present investigations the chief objection to the use of copper salts is that under the conditions adopted they form insoluble copper compounds with certain amino acids.

BLISH (**1**), working on wheat flour extracts, following a suggestion by OSBORNE and LEAVENWORTH (**20**), obtained very nearly complete separation. He treated the extracts with 0.1 N NaOH followed by 0.1 N $CuSO_4$ until the latter was only in slight excess of the equivalent required. Although his precipitates were free from amino groups, evidence was presented that peptide linkings of unknown nature but of less complexity than true proteins were still present in small amounts in the filtrates.

Both acetic acid and trichloroacetic acid are used by a large number of investigators both in the animal and plant physiological fields. The use of the latter was proposed by GREENWALD (**11**) because of its easy solubility, its ready volatility and its non-solvent action on proteins when used in excess. It is claimed that it does not precipitate either the proteoses or the peptones (**12**), although the results obtained in this investigation are inexplicable on this basis.

Colloidal ferric hydroxide was first introduced by RONA and MICHAELIS (**22**), since which time it has received more favor as a precipitant for blood protein than possibly any other reagent. The evidence presented by CLEMENTI (**8**) and also by VAN SLYKE, VENOGRAD-VILLCHUR and LOSEE (**29**) indicates that this reagent precipitates none of the amino acids; the latter also found that it precipitates none of the intermediary products up to the proteoses and none of these except some of complexity but little below that of the original proteins. Moreover, since the present investigations were completed, FODOR and REIFENBERG (**10**) have shown that the proteins adsorbed by the ferric hydroxide gel can be recovered completely by eluating the residuum with NaOH.

Mercuric salts have the advantage that they remove all pigments, giving colorless solutions; but the excess of mercury is not conveniently and easily removed. Like copper they also precipitate polypeptides.

Experimental

The effects of four of these reagents on the nitrogenous constituents of the water extracts of the leaves of *Pyrus Malus* were examined. The technique of precipitating the soluble proteins with the various reagents was as follows:

Acetic acid and trichloroacetic acid.—The extract (2,000 cc.), after the addition of 10 cc. of 10 per cent. acetic acid or of 25 cc. of a 10 per cent. solution of trichloroacetic acid, was heated to boiling and boiled one to two minutes. The coagulum was separated by a fluted filter paper, washed with 100 cc. hot water, and made up to the original volume.

Copper hydroxide.—To the extract, measuring about 2,000 cc. in a 4 liter flask, was added 400 cc. of a freshly made-up solution of 0.2 N NaOH, followed by 400 cc. of 0.2 N $CuSO_4$. Ten cc. portions of the 0.7 N $CuSO_4$ were then added, and after each addition the extract was well shaken and the precipitate allowed to subside. The copper-protein precipitate was removed by filtration and washed with water.

Colloidal ferric hydroxide.—Merck's colloidal ferric hydroxide was used. The extracts were brought just to the boiling point, when an excess of the reagent, determined by examining the filtrates for freedom from proteins, is added. In the present experiments 5–6 cc. is sufficient. The extracts were then brought to the neutral point and the boiling continued for one or two minutes. In partition experiments of the nature to be described, it is necessary to remove the excess of iron by the addition of $MgSO_4$ or some other salt of high coagulating power. From 0.5 to 1.0 cc. of a saturated solution of this salt is sufficient to produce coagulation of the iron gel. The precipitate of protein-ferric hydroxide gel is separated by filtration, the precipitate washed, and the filtrate made up to the original volume.

Determination of water-soluble protein nitrogen

In all cases the protein N was determined by difference between the non-protein N and the total water-soluble N. The large fluted filter necessary for the filtering required too large a blank to enable the small amount of protein to be determined directly with any degree of accuracy.

In table III the mean of closely agreeing duplicate determinations is given.

TABLE III

Comparison of protein N, non-protein N and amino N in the water extracts

Series no. OC	Nitrogen in the dry matter				
Precipitating reagent	Total N	Total water soluble N	Protein N	Non-protein N	Amino N
	Per cent. 1.83	Per cent.	Per cent.	Per cent.	Per cent.
Acetic acid	.....	0.144	0.034	0.110	0.014
Trichloroacetic acid	.....	0.146	0.034	0.112	0.018
Copper hydroxide	.....	0.146	0.078	0.038	0.008
Colloidal ferric hydrate	.....	0.142	0.022	0.120	0.024

QUALITATIVE TESTS ON THE FILTRATES FROM THE VARIOUS PRECIPITATING REAGENTS

The filtrates from the various reagents used for removing the proteins were subjected to the biuret, Millon and Adamkiewicz reactions. Such tests serve as a general guide, but caution must be used in drawing conclusions, for, as MOLISCH (**18**) has pointed out, the blue color of the biuret test is given also by certain carbohydrates and a number of aliphatic acids; hence, the test is not specific for proteins and the $-NH_2-CO-CO-NHR-$ groups. Neither can the Millon test be regarded as specific for the tyrosine and tryptophane groups of proteins, because it is also given by phenol, salicylic acid and other substances containing a monohydroxybenzene nucleus, upon the presence of which the test for proteins depends. The biuret test is best carried out by the method of KANTOR and GIES (**15**), who describe the preparation of a stable biuret reagent which is more sensitive than the classical method of application. The Adamkiewicz reaction was found in the present investigation to be more sensitive if the solution to be tested is concentrated almost to dryness *in vacuo*, followed by extraction with glacial acetic acid and concentrated HCl and not concentrated H_2SO_4 as usually recommended.

Some investigators (**10**) use SPIEGLER and ESSBACH's reactions to differentiate the derived from the non-derived proteins (*i.e.*, the simple and conjugate proteins free from their hydrolytic products). However, it is questionable (**14**) if these reagents are specific.

One of the difficulties encountered in applying such tests to plant extracts is the difficulty in removing all pigments present without also removing some of the products to be tested. The present tests were made on carbon black-treated extracts, which were concentrated *in vacuo* after filtering.

The results are conveniently shown in table IV.

TABLE IV

QUALITATIVE TESTS OF EXTRACTS FOR PROTEINS

REAGENT	FILTRATES	BIURET	ADAMKIEWICZ	MILLON
Copper salt	clear	negative	negative	slight positive
Acetic acid	turbid	positive	positive	positive
Trichloroacetic acid	turbid	positive	positive	negative
Colloidal ferric hydroxide	clear	positive	positive	positive

Qualitative tests, however, although devised for pure proteins and other decomposition products, may throw additional light on the results obtained by quantitative means and thus serve as an aid in interpretation.

Discussion

The quantitative results, table III, show that $CuSO_4$ and NaOH have removed more nitrogen from solution than the other reagents. This appears to be the result of the occlusion or formation of insoluble copper-amino-acid compounds (*e.g.*, asparagine) as well as to the complete removal of the specific peptides occurring in these extracts together with products intermediate between these and the true proteins. The negative biuret reactions in the copper filtrates give further confirmation of this, for a positive test is first encountered in certain tetrapeptides and is the more intense the greater the length of the polypeptide chain (**21**). This same test shows that the other reagents have not removed peptides and the intermediate products. The colloidal ferric hydroxide gel contains the lowest amount of nitrogen and the highest quantity of amino N. The presence of proteose N in the filtrates from the colloidal iron treatment may be objectionable from the standpoint of its influence on the determination of amide N, because of the possible error in the determination of amide N in the process of hydrolyzing the extracts. This was not investigated further because the amounts present are so small that the effect on the analytical results would be insignificant. The evidence to be presented in the third paper of this series shows clearly that all filtrates contain the CONH groupings.

Colloidal ferric hydroxide, then, is admirably suited for the separation of true proteins from solution because: (1) It is more convenient and expeditious; (2) it permits all the amino acids to go through, occluding and precipitating none; (3) it effects sharp separation of true protein from its decomposition products; and (4) the adsorbed proteins can be recovered quantitatively from the residuum.

Summary

Desiccation of the leaves and new wood growth from apple trees at 50°, 60° or 70° C. has no effect on the total nitrogen; but relatively small decreases in the soluble cytoplasmic proteins, and increases in the non-protein constituents, occur through drying. Considering the effect of temperature on both coagulation and proteolysis, it would appear that a temperature of 60° C. is the optimum for the desiccation of the tissues of this species, which belongs to the type of plants that are low in water-soluble protein nitrogen.

Colloidal ferric hydroxide has many advantages over the reagents in general use for the separation of the simple and conjugate proteins from their hydrolytic products. Reasons for this choice are given.

The writer takes this opportunity of expressing his thanks to Professors J. B. HILL and L. O. OVERHOLTS for microscopic examination of some of the extracted materials.

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NITROGENOUS METABOLISM OF *PYRUS MALUS* L.

II. THE DISTRIBUTION OF NITROGEN IN THE INSOLUBLE CYTOPLASMIC PROTEINS[1]

WALTER THOMAS

Before undertaking the experimental work on the distribution, throughout a year's cycle, of the soluble nitrogenous constituents of the leaves and branch growth, which will be presented in the third paper, it was considered of interest to obtain information respecting the nature of the insoluble leaf proteins and to ascertain what fluctuations, if any, occur during development, in the distribution of its nitrogen groups (*i.e.*, its amino acid make-up). An additional motive for a closer study of this insoluble portion was afforded by the relationships which NIGHTINGALE (**8**) seemed to find between his alcohol insoluble nitrogen fraction, and carbohydrates, in the vegetative and reproductive processes of the tomato plant (*Lycopersicum esculentum*).

Sufficient knowledge is not yet available to enable these insoluble proteins to be isolated in a state of purity, but VAN SLYKE (**11, 12**) has devised a method for determining the distribution of nitrogen in the various groups of the protein molecule which has furnished means of classifying different proteins by their amino acid make-up. This method was, however, devised for pure proteins and their hydrolytic products (**1, 2, 3**), but as MORROW and GORTNER (**7**) have pointed out, the extension of the method may be made to impure proteinaceous substances when due precautions are taken and no attempt is made to assign any part of the nitrogen to any specific amino acid.

Experimental

Samples of leaves were collected, from a 15-year-old Stayman Winesap tree growing in sod, at three critical physiological periods, *viz.* (1) at the early period of bud formation (May 13), (2) at the stage of active maximum growth (July 22), and (3) at the period of chlorophyl degeneration, *i.e.*, of declining metabolic activity (Nov. 11).

Five grams of each residuum, dried *in vacuo,* remaining after the extraction with water as described in the preceding paper and consisting, therefore, of the insoluble cytoplasmic proteins and the cellulose material of the cell wall, together with such non-protein nitrogenous materials as fats, lecithins, chlorophyl, and the purine and pyrimidine bases, were ex-

[1] Published by permission of the Director of the Agricultural Experiment Station as scientific paper no. 419 and contribution no. 25 from the Department of Agricultural and Biological Chemistry.

tracted with alcohol followed by ether for the removal of fats, chlorophyl and lecithins, and afterwards digested with 0.5 per cent. HCl (**10**) to hydrolyze the interfering carbohydrates (**5**). After filtering and washing with hot water, each residuum, consisting of the impure proteins, was hydrolyzed with 20 per cent. HCl for 48 hours on a sand bath, with the usual precautions, to insure comparable results (**4**). The small amount of protein N in the filtrate from the 0.5 per cent. HCl hydrolysis was separated and hydrolyzed after the process described by HAMILTON, NEVENS and GRINDLEY (**5**). After the removal of the HCl by distillation *in vacuo*, the two hydrolysates were combined. The residue was dissolved in 100 cc. of water and a slight excess of MgO added. The process of determining the nitrogen fractions was then, with slight modifications, similar to that described by MORROW and GORTNER (**7**).

The analytical data are presented in table I.

TABLE I

THE INSOLUBLE CYTOPLASMIC PROTEINS
DISTRIBUTION OF N AS PERCENTAGE OF TOTAL NITROGEN

NITROGEN FRACTION		SERIES NO. 3		SERIES NO. 20		SERIES NO. 33	
		(a)	(b)	(a)	(b)	(a)	(b)
Ammonia N	(A)	15.50	15.20	14.80	14.20	16.40	16.00
Insoluble humin N	(B)	7.82	8.00	7.75	7.70	9.00	8.80
Humin N precipitated by $Ca(OH)_2$	(C)	0.23	0.20	0.24	0.27	0.24	0.29
Total humin N	(A + B)	8.05	8.15	7.99	7.97	9.24	9.19
Total basic N	(D)	20.62	20.55	21.80	21.70	22.05	20.30
Basic N set free as NH_3 by 50% KOH (arginine)	(E)	5.80	lost	5.92	6.00	6.34	6.15
Basic N not set free as NH_3 by 50% KOH	(D − E)	14.82	.....	15.88	15.70	15.71	14.15
Amino N of bases	(F)	5.25	.....	.....	.....	.....	.....
Non-amino N of bases	(D − F)	15.37	.....	.....	.....	.....	.....
N in filtrate from bases	(G)	53.10	52.80	54.85	54.91	55.00	54.20
Amino N in filtrate from bases	(H)	51.30	51.00	53.20	.....	53.00	.....
Non-amino N in filtrate from bases	(G − H)	1.80	1.80	1.65	.....	2.00	.....
Total N obtained		97.27	.....	98.72	99.44	102.69	99.80

In series no. 33 (a) fats, lecithins, chlorophyl, etc., were removed by previous extraction with alcohol and then with ether. This would contain a small amount of nitrogen, but the total amount as compared with the protein N present is small.

Discussion and conclusion

Is it justifiable to draw from the constancy of the distribution of nitrogen the conclusion that, although the leaf proteins vary quantitatively through-

out their cycle, they do not vary qualitatively? In other words, do these results indicate that a single protein exists in the leaves or do they contain a mixture of several? OSBORNE (**9**) believes that even if several proteins occur in these leaves they may be so nearly alike in their amino acid make-up that changes in their relative proportions can not be established by methods at present available. He is of the opinion that, though the above results do not give *absolute proof*, perhaps, that a single protein is present in these leaves, they do *indicate* that this is the case.

Even if these "insoluble" proteins could be isolated in the pure condition so that not only the percentage of amino acids but also their optical properties, molecular weight and elementary composition could be determined, the similarity of all these would not constitute indisputable evidence that the proteins were identical. If marked differences occurred in the amino acid content there would be little question as to the existence of a mixture of substances of unlike chemical entities; but these results, until new methods of study become available that enable the question of the true chemical entity of the proteins to be determined, do indicate that little, if any, qualitative change in the nature of the insoluble leaf protein has taken place during its development.

From this standpoint, then, it is clear that any relationship to plant performance that may be discoverable between the insoluble proteins and carbohydrates must be due to quantitative and not qualitative differences in the nature of the insoluble proteins.

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SOIL-MOISTURE CONDITIONS IN RELATION TO PLANT GROWTH

F. J. VEIHMEYER AND A. H. HENDRICKSON

(WITH SIX FIGURES)

Recent investigations at the University of California have yielded much information regarding the relation of soil moisture to the normal growth and behavior of fruit trees, and have indicated serious disagreements with results secured by some previous investigators of the problem of water relations of plants. It is logical to suppose that the moisture conditions of the soil do not exert any direct retarding influence on the plant so long as water is supplied to the absorbing surfaces of the roots and is conducted to the leaves as rapidly as required by the transpiration rate. However, just as soon as the rate of supply falls below this requisite amount, then soil moisture becomes a limiting condition. An inadequate moisture supply may be evidenced by lessening of turgor, cessation of growth, and wilting, and, in more advanced stages, by death of the tissues. Of course, the wilting of a plant does not indicate that water has ceased to move from the soil into the plant, but simply that transpiration has exceeded absorption and conduction. It is obvious, since wilting is progressive, that various stages of wilt might be recognized. Several attempts have been made to fix upon a definite degree of wilting. The "saturation deficit" of RENNER (**6**) and the "incipient drying" of LIVINGSTON and BROWN (**4**) are not definite stages, but represent broad ranges in the progress of wilting, but the "permanent wilting" of BRIGGS and SHANTZ (**1**) represents a fairly definite stage or degree of wilting, which can be readily recognized in experimentation, and this has received much study. However, KOKETSU (**3**) has pointed out that definite stages of wilting may be recognized readily in *Mimosa* because of the movements of its leaves.

BRIGGS and SHANTZ defined their permanent wilting as that stage of wilting when the leaves first undergo a permanent reduction of their moisture content as a result of deficiency in the soil-moisture supply. A permanent reduction is here taken to mean a deficiency in leaf-water content from which the leaves do not recover in an approximately saturated atmosphere, without the addition of water to the soil. LIVINGSTON and KOKETSU (**5**) have further discussed the condition of permanent wilting and have emphasized the dynamic nature of the wilting process.

BRIGGS and SHANTZ (**1**) concluded from their studies that atmospheric environmental conditions have little or no effect upon the residual water content of the soil at the time of the beginning of permanent wilting and

that this residual water content, called by them the wilting coefficient, for any given soil is a constant for all species of plants grown on it and for all stages of their development. On the other hand, CALDWELL (**2**) and SHIVE and LIVINGSTON (**8**) found, from a study of plants grown in small containers, that the time required for the onset of permanent wilting after watering had been discontinued, and also the residual water content of the soil when permanent wilting began was dependent upon the intensity of the evaporation conditions for the period during which permanent wilting was attained. LIVINGSTON and KOKETSU (**5**) found that the water-supplying power of the soil was practically constant for all soils when permanent wilting began in their plants, and they predicted that this critical value of the supplying power would be found to depend upon the evaporation conditions.

Observations extending over a number of years in deciduous fruit orchards in California, as well as in experimental plots at Davis, California, at the Branch of the College of Agriculture of the University of California, indicate that the soil-moisture supply may fluctuate between wide limits without measurably affecting the growth of the tree or the yield and quality of the fruit. This range of fluctuations in the moisture of the loam soils on which the experiments were conducted was between the maximum field capacity and the calculated wilting coefficient, a range of 10 per cent. or approximately 9.5 acre-inches of water in six feet of soil. The difficulty of adequately sampling the soil in the orchard plots, in spite of the employment of an unusually large number of samples, and the consequent difficulty of determining with satisfactory certainty the average soil-moisture condition in the root zone, led to a study of trees grown in large containers, and the results from these experiments were substantially the same as those obtained in the orchard plots.

Experimental results based on the idea that water applied at any point in the soil would be quickly and uniformly distributed throughout the surrounding soil have led, in the opinion of the writers, to many erroneous conclusions. This thought has also recently been put forth by others, notably SHANTZ (**7**), who stated upon theoretical considerations that "Because of these peculiarities in the distribution of moisture in soils much of the work . . . is entirely unreliable and will have to be repeated when the conditions are known or better understood." The writers have been unable to maintain any soil-moisture content lower than that which the soil would hold against the force of gravity—the maximum field capacity. What takes place when water is applied to the surface of nearly dry soil is illustrated by the results of a test, the results of which are shown in figure 1. A rain of 2.15 inches on the surface of a loam soil resulted in wetting the soil to a uniform depth of about 14 inches, but no farther, samples being taken 48

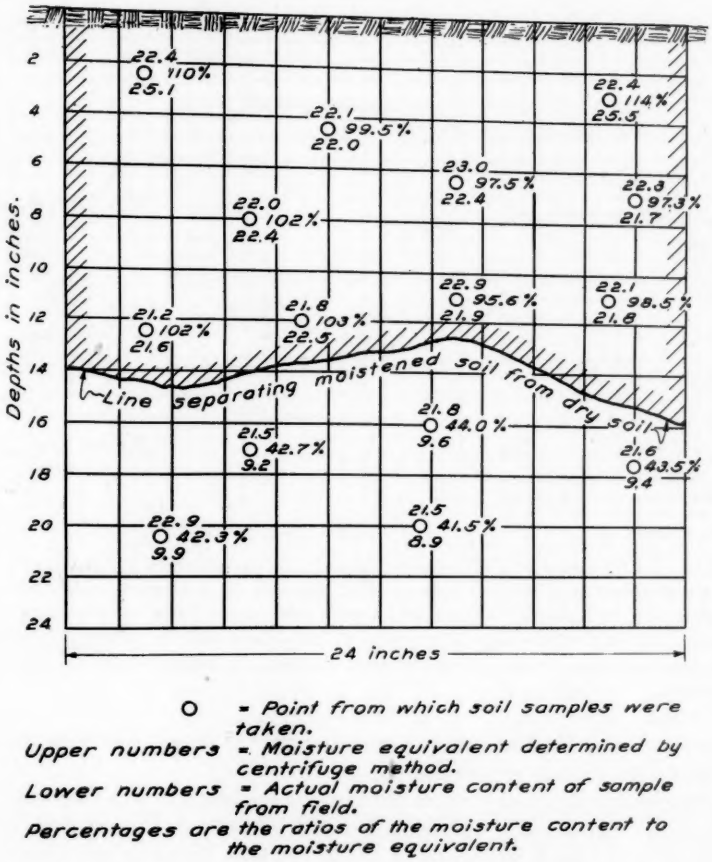

FIG. 1. Vertical distribution of water in a loam soil 48 hours after a rainfall of 2.15 inches. The upper margin of the diagram represents the soil surfaces.

hours after the rain ceased. As the figure shows, the soil throughout the wetted region was raised to a moisture content closely approximating its moisture equivalent made by the method suggested by VEIHMEYER, ISRAELSON and CONRAD (9). In general, an application of a definite amount of water on the soil surface results in the wetting of the loam soils used in these experiments always just to a definite depth, this depth depending upon the water-holding capacity of the soil and its initial moisture content. This was observed many times in our work, both in field plots and in large con-

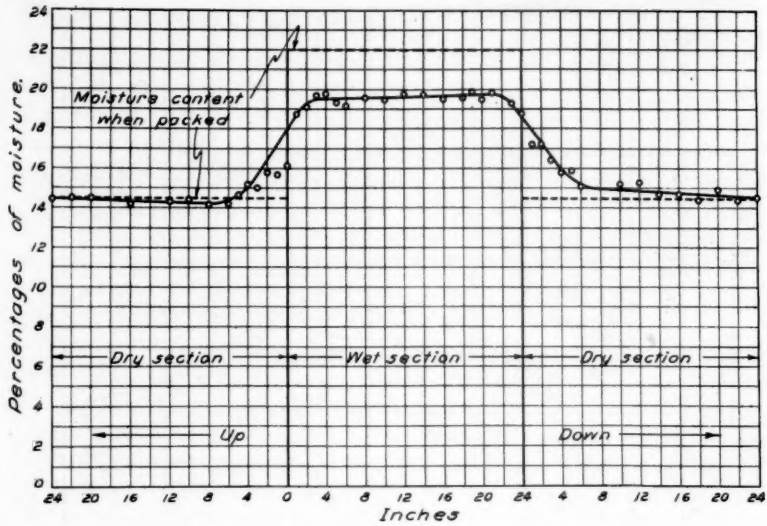

FIG. 2. The movement of moisture upward and downward (left and right in diagram) from soil mass initially containing 22 per cent. of moisture to soils containing 14.5 per cent. of moisture. Column was started September 2, 1922; samples were taken January 18, 1923. The place of sampling in the column and the amount of moisture found are indicated by the circles. Depth is shown by numerals along the base of diagram.

tainers. There is no evidence in such tests of the deeper soil being wetted at all by the surface application.

The results obtained in these experiments are contrary to a long accepted belief that water moves by capillarity with considerable speed from moist to drier soils. Such movement as occurs has been found in our tests to be extremely slow in rate and slight in both amount and extent. The data presented in figure 2 show the movement of moisture upward and downward within a period of 139 days, from a soil mass initially containing 22 per cent. of moisture, calculated on a dry weight basis, to soil masses above and below containing 14.5 per cent. of moisture. The column of loam soil had a cross-sectional area of 36 square inches. The central wet section was initially packed with soil containing water to the extent of its maximum field capacity (22 per cent.) and the upper and lower end sections were packed with the same soil but with moisture content somewhat above the calculated wilting coefficient. The extent of movement in either the upward or the downward direction was approximately 8 inches in the period of 139 days.

Like results were obtained in many similar trials, with soils of different initial moisture contents.

In the experiments with trees in containers, at each application of water the soil mass was raised to its maximum field capacity throughout. The trees were then allowed to deplete the average soil moisture to different extents and then water was again applied. The range of moisture fluctuations was slight, in some cases, while in others it was as much as from the maximum field capacity to the calculated wilting coefficient. These experiments continued through the growing season. The use of water by young French prune trees grown under these conditions of fluctuating soil moisture supply is shown by the data in table I.

TABLE I

AMOUNT OF WATER USED BY YOUNG FRENCH PRUNE TREES, AS RELATED TO LEAF AREA AND TO LENGTH GROWTH

NUMBER OF TREE	LENGTH GROWTH	NUMBER OF LEAVES	LEAF AREA	WATER USED MARCH 1 TO SEPTEMBER 25	RATIO OF WATER USE TO LEAF AREA (LOSS PER SQUARE INCH OF AREA)	RATIO OF LENGTH GROWTH TO WATER USE (INCHES OF GROWTH PER POUND OF WATER USED)
	inches		sq. in.	pounds	pounds	pounds
5	348.0	394	2098	499	0.237	0.698
12	214.0	239	1244	316	0.254	0.678
20	697.5	828	4305	1020	0.237	0.683
21	351.0	398	2070	508	0.245	0.690

For tree no. 5, the soil-moisture content was kept above 16 per cent. until the middle of August, being then alternately allowed to fall to approximately the wilting coefficient, and irrigated; for tree no. 12, the soil was kept water logged throughout the experiment; for tree no. 20, the soil-moisture content was maintained above 16 per cent.; and for tree no. 21, the soil-moisture content fluctuated between the maximum field capacity and an amount corresponding to the wilting coefficient. The maximum field capacity (or the moisture equivalent) for this soil was approximately 22 per cent. The use of water was nearly proportional to the leaf area and still more nearly proportional to the growth in length. The coefficient of correlation between the use of water and the leaf area for all the trees used (of which those given in table I are a part) is 0.97 ± 0.11, and that between use of water and growth in length is 0.995 ± 0.002. There is apparently no relation between soil-moisture content and either use of water or growth in length as here shown.

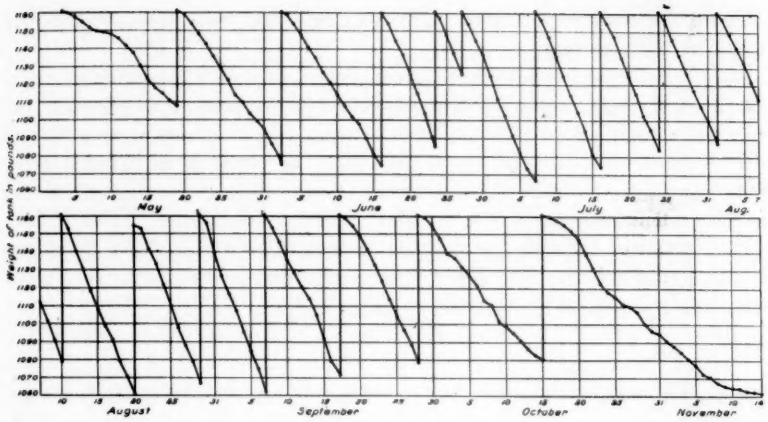

FIG. 3. Use of water by prune tree during the season of 1922, at Mountain View. The weight of the tank with soil containing 22 per cent. of moisture was 1161 pounds and the weight when the soil was at the wilting coefficient (11.9 per cent.) was 1070 pounds.

A further example to show that the rate of use of water by young prune trees in containers was not affected by the amount of water in the soil, provided the moisture content was not below the calculated wilting coefficient, is shown by the graph of figure 3, which presents data which were obtained from a tree on an automatically recording balance. The vertical portions show the applications of water, and the slope of each intervening portion of the graph indicates the rate of use of water, which was clearly no greater with high than with low soil-moisture contents.

When the soil-moisture content was reduced to a percentage corresponding approximately to the calculated wilting coefficient, the trees wilted and did not revive until water was applied to the soil. A remarkable degree of agreement between the observed and the calculated wilting coefficient is shown in table II in a number of cases, which are representative of a much larger number, for two different localities, and at different times during the growing season.

Still further evidence of the importance of the wilting coefficient as a critical point in the process of soil-moisture depletion by plant transpiration was secured from studies on the width of stomatal openings. Apricot, prune and peach trees growing on soil with water content higher than that corresponding to the wilting coefficient showed markedly wider stomatal openings by day than did those grown on soil with water content at or near the wilting coefficient. The average results obtained with prune trees during a 24-hour period beginning at 6 A. M., September 11, 1924, are shown

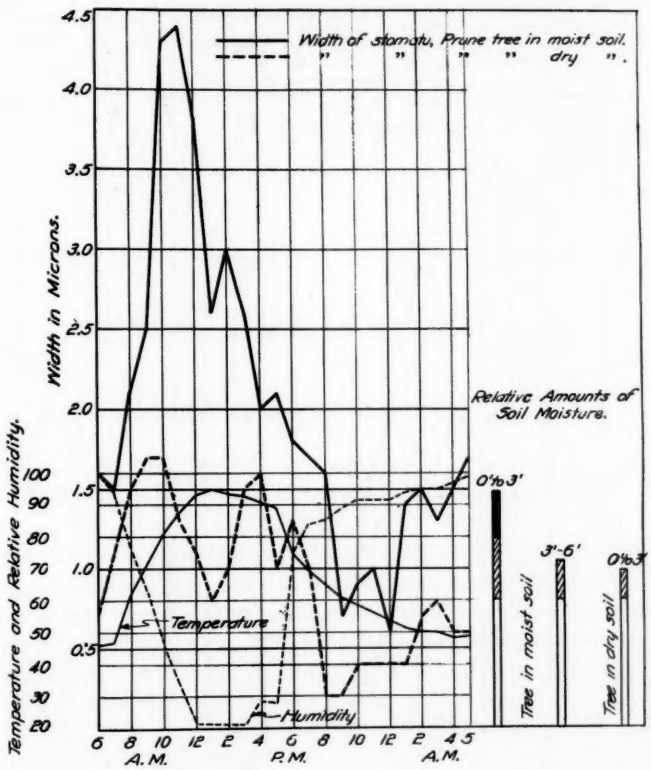

FIG. 4. Width of stomatal opening on prune trees in moist soil and in dry soil at Mountain View, California, September 11, 1924. Temperature and relative humidity are shown by the light lines in the lower left corner. Relative amount of soil moisture above the wilting coefficient is shown by the solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

graphically in figure 4. The usual diurnal variation in stomatal widths is shown for both moist and dry soils, but the average maximum width for the moist soil is seen to have been about three times as great as for the dry soil. Similar results obtained with peach trees under climatic conditions essentially the same as those to which these prune trees were subjected are shown in figure 5, along with a similar graph for another prune tree on moist soil. However, differences in width of stomatal openings of leaves on any of the trees studied could not be detected when the soil-moisture contents varied but did not fall to the wilting coefficient. The average measurements of

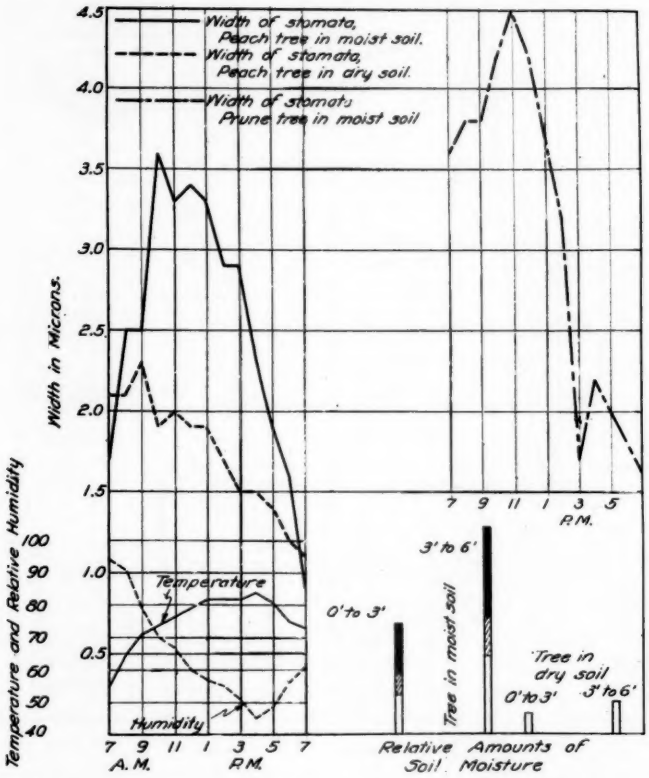

FIG. 5. Width of stomatal opening on peach and prune trees in moist soil and peach tree in dry soil at Delhi, October 1, 1924. Temperature and relative humidity are shown in lower left corner. Relative amount of soil moisture above wilting coefficient is shown by solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

stomata of leaves on peach trees, one of which was on a soil with high moisture content, and the other on a soil with low moisture content but still above the wilting coefficient are shown graphically in figure 6. The graphs show that no real differences exist in width of stomatal opening. The stomata appear to have behaved as usual as long as the soil-moisture content was higher than the wilting coefficient. These results also substantiate the data given in table I, in that the trees were not affected until the soil moisture was reduced to the wilting coefficient.

TABLE II

RELATION OF THE OBSERVED TO THE CALCULATED WILTING COEFFICIENT FOR YOUNG PRUNE TREES IN CONTAINERS

TREE NUMBER	DATE WILTED	SOIL-MOISTURE CONTENT AT TIME OF WILTING	WILTING COEFFICIENT CALCULATED FROM MOISTURE EQUIVALENT
		per cent.	per cent.
3	May 5, 1922	11.6	11.1
3	August 14, 1922	10.5	11.1
3	September 28, 1922	11.2	11.1
5	August 25, 1922	13.0	12.7
5	October 7, 1922	12.7	12.7
5	May 3, 1923	12.3	12.7
6	August 21, 1922	11.6	11.9
6	September 2, 1922	11.7	11.9
6	April 29, 1922	11.3	11.9
19	June 13, 1924	11.1	11.2
20	June 12, 1924	11.0	11.4
10	July 7, 1924	11.9	11.6

Trials with mature peach and prune trees showed that growth in length of shoots could not be prolonged indefinitely by maintaining high soil-moisture contents throughout the season, and the same result was secured with young trees in containers. If the average percentage of water in leaves and bark and wood of all parts of the tree may be used as a criterion of maturity the results obtained with bearing peach trees indicate that trees growing on wet soils matured at the same time as trees on nearly dry soils for the mean water content of these parts of the tree was approximately the same in the autumn for both soils. Furthermore, young trees on moist soil held in containers dropped their leaves at the same time in the fall as those on drier soils. However, defoliation could be brought about during periods of high evaporating conditions by withholding water until the trees wilted.

The data secured in these studies seem to have an important bearing on a number of questions regarding the relation between irrigation and the hardening or maturing of the wood and buds of fruit trees. An abundant supply of soil moisture throughout the season can not alone account for the immaturity of the current growth of the tree and so-called winter injury that seems to be a result of such immaturity, at least under the conditions of these experiments.

The correlation between leaf area and the use of water by trees is illustrated in a concrete way by results obtained in one of the orchards at the Branch of the University of California at Davis. This orchard consisted

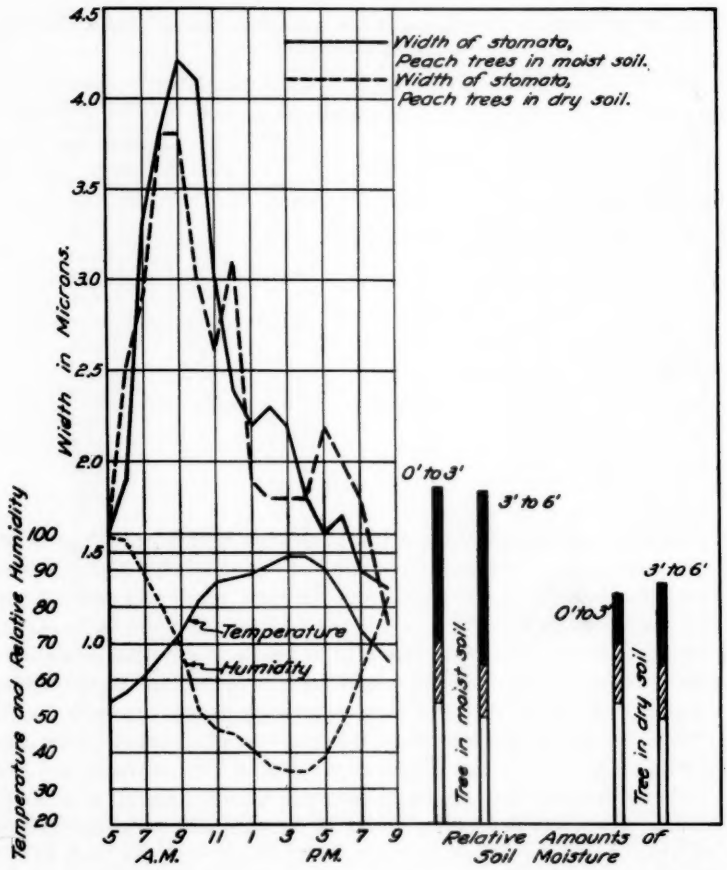

FIG. 6. Width of stomatal opening on peach trees on moist soil and on dry soil at Davis, California, July 9, 1925. Temperature and relative humidity are shown by light lines in lower left-hand corner. Relative amount of soil moisture above the wilting coefficient is shown by solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

of suitable plots of cherries, plums, prunes, pears, peaches, and apricots, with different spacings. Thus, in the first block the trees were 12 feet apart, in the second, 16 feet apart, and so on until the trees of the last block were 36 feet apart. The trees were planted in 1915 and at the present time only those in the 30-foot and 36-foot blocks are alive. The trees in

the 12-foot block showed evidence of drought during the summer of the fourth season. After the fourth or fifth year growth was less and injury (such as sunburn) was greater in the more closely planted blocks than in the others. Those of the 12-foot block showed signs of drought, as evidenced by curling and dropping of the leaves, from two to five or six weeks before those of the 24-foot block showed these symptoms. The greater leaf area, in proportion to the moisture available to the trees, in the closely planted blocks was probably directly concerned with the premature decline of these trees.

The relation of leaf area to use of water by the tree has an important bearing on irrigation practice, especially when alfalfa is grown in the orchard as a continuous cover crop. The combination of trees and alfalfa requires more water than do trees alone. The writers are of the opinion that any benefits derived by the trees from the growing of alfalfa in this way are probably due to causes other than lessened transpiration, on the part of the tree, though this cause is often given to explain benefit to the trees apparently due to the cover crop.

The writers wish to express their thanks to Dr. B. E. LIVINGSTON, of Johns Hopkins University, who kindly read the paper and offered many helpful suggestions.

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DOES THE PEA PLANT FIX ATMOSPHERIC NITROGEN?

DEAN BURK

LIPMAN and TAYLOR (3) several years ago reported the fixation of atmospheric nitrogen by wheat and barley plants grown in culture solutions. Preliminary experiments with peas grown under similar conditions did not, however, show a similar power of fixation. These authors stated that their results with peas were inconclusive, since too small a number of plants were grown and statistical methods were not employed. They recommended that further studies be made. In their experiments only twenty one-liter jars, each containing three pea plants, were employed; no attempt was made to maintain sterile conditions; the culture solution used was tap water of unknown composition except for its nitrogen content, which was 1.0 mg. per jar; and since no statistical method was employed, the nitrogen contents of the seeds were not very accurately known. The following significant changes and improvements have been made in the experiments reported here. An adequate statistical method for the interpretation of analyses of seeds and harvested plants has been employed; sterile conditions have been maintained up until the moment of planting; two series were run, each differing with respect to the amounts of nitrogen supplied beyond that contained in the seed; the solutions were made up with distilled water and definite amounts of chemicals, and were analyzed for nitrogen both before and after the plants had grown in them; and at the end of the growing period the residual solutions were tested qualitatively for the traces of nitrogen contained in them.

Experimental

The general experimental procedure was much the same as that employed by LIPMAN and TAYLOR. The Dwarf variety of *Pisum sativum* was used. There were two plants in each jar, the jars having a capacity of two liters. The solutions of the two series, differing from each other only with respect to the amounts of nitrogen, and this in the form of nitrate, were as follows: The salts are expressed as mols, the water as liters, and the concentration as molality.

Series	KH_2PO_4	$MgSO_4$	$CaSO_4$	$Ca(NO_3)_2$	H_2O	Total concentration
1 No nitrate	0.412	0.364	0.120	none	up to 40	0.0224
2 Nitrate added	0.412	0.364	0.120	0.012	up to 40	0.0227

The salts were added from their molal solutions, except $CaSO_4$, in which case 8.5 liters of a saturated solution were used. It was assumed that this

volume contained .120 mol. The solutions were analyzed for nitrogen by both the Devarda and modified Gunning-Kjeldahl methods (Davidson scrubbers were used), using three 200 cc. aliquots of each, and running four blanks on the chemicals with distilled water. The net totals of milligrams of nitrogen for each solution per jar were: Solution 1, 0.0 mg., solution 2, 8.0 mg., with an accuracy of $\pm$ 0.2 mg. Baker's analyzed chemicals without purification were employed. Two liters of solution were poured into each of the Mason jars, covered with a large amount of cotton, which was then tightly bound on, and sterilized for an hour in the autoclave at 15 pounds pressure. The jars had been previously cleaned twice with commercial concentrated hydrochloric acid and then rinsed thoroughly with distilled water. They were covered with paper on the outside. New two-hole corks which would fit tightly into the jars were paraffined and sterilized in the same manner as the jars. They were fitted into the jars at the moment of planting. The Dwarf pea seeds were selected to a size of 21 $\pm$ 1 centigrams and were sterilized as follows. They were placed in $HgCl_2$ (1/5000) for five minutes and then in sterilized distilled water for one hour and fifty-five minutes, a method suggested by Dr. LIPMAN. This operation was repeated twice. They were then placed as aseptically as possible in a large ten-inch sterilized Petri dish and incubated at 28 degrees for four days, during which time no visual trace of contamination by microorganisms developed. Sterile jars, solutions, corks, and seedlings were then taken to the greenhouse where the planting was done and where the plants remained throughout their entire growth period, which was 58 days from planting until harvest (September 27 to November 24). The peas were fitted into the holes in the corks with the assistance of sterilized cotton, which also served as a protection for the solution from the dust of the air. The only additional substance added to each of the jars was 5 cc. of a 0.5 per cent. solution of $FeSO_4$, as follows: 1 cc. on the 18th day after planting, 2 cc. on the 25th day, and 2 cc. on the 30th day. No additional water was added. After harvesting, the plants of each jar were separately dried at 96° C. for 26 to 38 hours, weighed, and analyzed for total nitrogen by the modified Kjeldahl method. The digestion was carried on for a full three hours. An aliquot of 500 cc. of the total solution remaining in each of the jars was analyzed in a similar manner. As these solutions were found by qualitative tests to contain no nitrate, the Devarda method was not employed in their analysis. Dilute N/50 standard acid and alkali were used when analyzing the solutions; in all other cases, N/10 solutions. Hence the limits of accuracy, on a basis of 0.1 cc., are 0.03 and 0.1 mg. respectively. Sufficient reagents for all Kjeldahl analyses were prepared in one large lot. The nitrogen content of these reagents was determined by a sufficient number of blanks. At the commencement of the experiments the original lot of seeds

(21 ± 1 cg. each) was analyzed by the modified Kjeldahl method in 15 sets of 2 each. Also fifteen sets of two seedlings each were analyzed. These seedlings were sterile and were six days old, two days older than those which were planted. The purpose of analyzing the seedlings was to determine if any loss of nitrogen occurred during germination. In determining the statistical mean of the nitrogen contents of the seeds, the results for seeds and seedlings, after being calculated separately for purposes of comparison, were then calculated together, since by so doing the accuracy of the values of both the arithmetical mean and the probable error was increased.

The usual statistical methods have been employed. The probable error is taken to be the product of the constant, 0.6745, and the standard deviation, all divided by the square root of one less than the number of variants. The standard deviation is the square root of the quantity obtained by dividing the sum of the algebraic squares of the deviations from the arithmetical mean by the total number of variants. The probable error of the difference between two means is the square root of the sum of the squares of the probable errors of each mean. The "minimum difference required for significance" is calculated by multiplying the probable error of the difference of the means by the usual factor 3.2. This calculated minimum difference when compared with the actual difference obtained shows immediately the exact quantitative value of the experimental results.

The following observations were made during the growth period. The number of days refers to the number of days after planting. At 21 days, the lower portions of plants of series 1 were dying. At 25 days, the lower portions of all plants of series 1 were apparently dying or dead, and about one sixth of the plants of series 2 were similarly affected. At 26 days, one third of the plants of series 2 were affected. At 30 days, flowering had commenced, mostly in plants of series 2. At 39 days, the entire leafage of series 1 was practically dead, becoming rather suddenly so over a period of four days. The flowering plants were the most alive. Nearly all the green portions of series 2 were dying also. At 42 days, all plants of series 2 and one half those of series 1 were sending forth new shoots on the lower portions of the tops. At 51 days, increasing green growth in series 2, but in series 1 the new green parts were dying again. At 56 days, the plants of every jar in series 2, except three, were alive, green, and healthy; the plants of every jar in series 2, except two, were without green foliage of any sort. The solutions of all jars of both series when tested with the diphenylamine reagent showed that there was less than one part in ten million of nitrogen as nitrates; and as the tests were all negative, there presumably were no nitrates. As this reagent detects various forms of oxygen-nitrogen compounds such as nitrites, nitro compounds, and others, it may demonstrate their absence also. Tested for ammonia by Nessler's reagent (and therefore

for many other amines also) all solutions, regardless of series, which contain living plants gave absolutely negative tests; and all solutions, regardless of series, in which the tops have died, showed the presence of small amounts of ammonia. This amount however, when the volume of solution is taken into consideration, was 1 to 2 mg. per jar, and is therefore of significant proportions. The roots of the plants gave no evidence of nodules. There had been no aphids on any of the plants. Only a few of the plants had mold. In general, the ratio of root to top was approximately constant, that is, the larger the top, the larger the root. At 58 days, plants were harvested and every root examined for nodules. None were found. The majority of plants had flowered; sixteen had produced pods containing seeds; the water transpired had been small, about 800 cc. per jar in series 1 (quite uniformly) and 800 cc. to 1,000 cc. in series 2. In none of the plants had there been any mortality during the first three weeks of growth.

The analyses for the nitrogen-free solutions, and plants grown in them, are given in table I. Those for solutions containing nitrogen are shown in table II. The nitrogen content of seeds and seedlings six days old is given in table III, and the results are summarized statistically in table IV.

Discussion

Within each series, the dry weights of the plants were quite closely proportional to the nitrogen contents, in accordance with the law of the minimum. Under these conditions of nitrogen starvation the pea plant showed marked avidity for ammonia and nitrate in the culture solution.

The fact that, in LIPMAN and TAYLOR'S experiments most of the plants lived, while in series 1 of these experiments most of the plants died, might possibly be explained either by the difference of the critical 1 mg. of nitrogen in the culture solution or by traces of an essential element which the tap water may have possessed. The death of plants of series 1, from all outward appearances, was due to physiological rather than to parasitic causes.

The presence of excreted nitrogen in the culture solutions, together with the very probable and generally accepted fact that plants may lose nitrogen gas during photosynthesis (early shown by the researches of SIR HUMPHREY DAVY, DE SAUSSURE, RIGG (**4**), DAUBENY (**1**), and DRAPER (**2**), make it possible that the pea plants in these experiments lost enough nitrogen to hide any evidence of fixation. See also SPOEHR (**5**) for a recent discussion of nitrogen loss during photosynthesis. Such a definite explanation has often been overlooked in the reports of the few experiments which have been performed along lines similar to these.

These experiments are an admirable inferential check on the positive experiments of LIPMAN and TAYLOR with wheat and barley. They indicate, first, the probable absence of bacterial intervention, and second, the general

TABLE I

SERIES 1. NITROGEN FREE SOLUTION

JAR NUMBER	DRY WEIGHT	N IN RESIDUAL SOLUTION	TOTAL N IN PLANTS AND SOLUTION	DEVIATION FROM MEAN
	cg.	mg.	mg.	
1	50	1.05	15.54	0.42
2	43	1.77	15.54	0.42
3	36	1.17	16.10	0.98
4	55	1.17	14.14	−0.98
5	47	1.05	14.84	−0.28
6	47	1.17	14.14	−0.98
7	47	1.50	lost	.....
8	55	1.71	16.52	1.40
9	53	1.05	13.72	−1.40
10	43	1.65	15.40	0.28
11	47	1.98	17.78	2.66
12	40	1.05	13.44	−1.68
13	80	0.66	19.46	4.36
14	46	1.11	16.24	1.12
15	43	1.50	14.70	−0.42
16	50	0.78	14.66	−0.46
17	56	1.05	16.52	1.40
18	36	1.17	13.30	−1.82
19	41	0.66	14.52	−0.60
20	47	1.65	15.40	0.28
21	25	1.05	10.36	−4.76
22	46	0.99	14.14	−0.98
23	52	0.84	14.84	−0.28
24	40	1.98	13.16	−1.96
25	50	1.44	16.48	1.36
26	40	1.44	13.96	−1.16
27	34	1.32	12.18	−2.94
28	47	1.05	16.24	1.12
29	40	0.78	15.96	0.84
30	58	0.60	19.18	4.06
—	—	—	—	—
Mean	46.4	1.21	15.12	
Probable error	1.2		0.24	

Volume of residual solution, 1200 ± 50 cc. in every case.
Only two jars where plants had green tops, 13, 19.
Only two jars with no ammonia in solution, 13, 19.
Plants larger than others, 4, 6, 8, 9, 13, 22, 30.
Pods on, 13.

TABLE II

SERIES 2. SOLUTION WITH NITRATE, 8 MG. N PER JAR IN SOLUTION

JAR NUMBER	DRY WEIGHT	N IN RESIDUAL SOLUTION	TOTAL N IN PLANTS AND SOLUTION	DEVIATION FROM MEAN
	Cg.	mg.	mg.	
31	82	0.21	21.14	−0.41
32	60	0.66	lost	
33	59	0.06	21.56	0.01
34	68	0.00	21.56	0.01
35	38	0.00	14.56	−6.99
36	73	0.06	22.40	0.85
37	87	0.06	22.96	1.41
38	72	0.36	18.34	−3.21
39	72	1.08	20.58	−0.97
40	72	0.30	21.98	0.43
41	60	0.63	21.84	0.29
42	72	0.42	20.82	−0.73
43	69	0.00	21.98	0.43
44	69	0.30	19.46	−2.09
45	75	0.36	20.82	−0.73
46	84	0.06	21.98	0.43
47	71	0.06	21.14	−0.41
48	90	0.21	21.00	−0.55
49	66	0.06	20.40	−1.15
50	70	0.06	17.36	−4.19
51	104	0.21	20.82	−0.73
52	75	0.03	20.82	−0.73
53	97	0.03	24.36	2.89
54	102	0.78	24.08	2.53
55	78	1.08	23.94	2.39
56	91	0.72	24.22	2.67
57	92	1.08	22.54	1.99
58	95	0.45	23.94	2.39
59	75	0.06	27.16	5.61
60	83	0.06	22.68	1.13
—	—	—	—	—
Mean	73.4	0.31	21.55	
Probable error	1.8		0.30	

Volume of residual solution (± 50 cc.) 1200 cc., 32, 33, 35, 39; 1000 cc., 54; 1100 cc., the remaining jars.

Only jars without green tops, 35, 39, 50.

Only jars with ammonia in solution, 35, 39, 50.

Plants smaller than others, 35.

Pods on, 32, 40, 41, 43 (two), 44, 47, 48, 51, 53, 54, 57, 58, 59.

TABLE III

NITROGEN CONTENT OF SEEDS, AND SEEDLINGS SIX DAYS OLD

SEEDS			SEEDLINGS		
SET NUMBER	N	DEVIATION FROM MEAN	SET NUMBER	N	DEVIATION FROM MEAN
	mg.			mg.	
1	16.24	−0.43	1	15.96	−0.47
2	18.48	1.81	2	16.10	−0.33
3	15.82	−0.85	3	16.38	−0.05
4	16.80	0.13	4	15.26	−1.17
5	17.22	0.55	5	15.68	−0.75
6	16.82	0.13	6	16.66	0.23
7	15.96	−0.71	7	17.50	1.07
8	14.70	−1.93	8	15.26	−1.17
9	18.62	1.95	9	19.60	3.17
10	18.62	1.95	10	19.20	1.77
11	16.84	0.17	11	14.70	−1.73
12	15.54	−1.13	12	17.78	1.36
13	18.86	2.19	13	13.86	−2.57
14	14.66	−2.01	14	17.54	1.11
15	15.82	−0.85	15	15.82	−0.61
Mean	16.67			16.43	
Probable error	.242			.257	

Seeds and seedlings together: Mean 16.55; probable error 0.19.

TABLE IV

STATISTICAL SUMMARY OF RESULTS

DETERMINATIONS	SERIES 1		SERIES 2	
	N	DRY WEIGHT	N	DRY WEIGHT
	mg.	Cg.	mg.	Cg.
Original solution	.0		8.0	
Original seed	16.55 ± 0.19	42.	16.55 ± 0.19	42.
Total initial	16.55 ± 0.19	42.	24.55 ± 0.19	42.
Total final	15.12 ± 0.24	46.4 ± 1.2	21.55 ± 0.30	73.4 ± 1.8
Probable error of difference of means	± 0.30		± 0.36	
Loss during growth period	1.43 ± 0.30		3.00 ± 0.36	
Gain during growth period		4.4 ± 1.2		31.4 ± 1.8
Minimum difference required for significance	0.96		1.15	
Per cent. gain or loss	−9.6	+10.5	−12.2	+74.8

sufficiency of their technique, in a manner, it may be added, which would be hard to equal.

Summary

The Dwarf variety of *Pisum sativum* when grown under the general conditions stated, either in the absence or in the presence of additional nitrogen in the original culture solution, and with sterile conditions maintained up until the moment of planting, has shown upon statistical treatment of the experimental results a small unqualifiable *loss of nitrogen*. This nitrogen was not lost during germination, but afterwards, during the growing period of the plant.

The writer wishes to express appreciation of suggestions offered by Dr. CHARLES B. LIPMAN.

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THE DETERMINATION OF POLYSACCHARIDES[1]

Because of the complexity of the polysaccharides, our knowledge of them is much less definite than our knowledge of the simple sugars, and this is strikingly reflected in the relative indefiniteness of the methods for determining them. In most cases, they need to be hydrolyzed to simpler substances as a preliminary step. Since the hydrogen ion is the universal catalyst for these hydrolyses, and since in most cases several polysaccharides occur concomitantly, the decided lack of specificity of determinations is evident. The most hopeful direction of improvement is in the development of biochemical methods, involving the use of specific microorganisms and enzymes.

In the following discussion it has been the aim of the committee to indicate the methods which are available for determining a given substance, and, where possible, to suggest the preferred one. Working directions are not given since the original citations are readily available, and since in many cases the committee's choice of a method may not be the one adopted by the investigator.

Cellulose

The determination of true cellulose is a laborious procedure, and has been applied almost exclusively to the analysis of wood and paper. The one usually used is the chlorination method of CROSS and BEVAN, as perfected by SCHORGER (**19**). Its success depends largely upon the proper grinding of the material. Twigs have been ground very handily in a pencil sharpener (**11**).

The old method for crude fiber, used in the proximate analysis of feeds, is at times useful in physiological investigations (**28**). It gives a rough idea of the cellulose of the tissues, and is much more easily carried out than the method for true cellulose.

Starch

Preliminary treatment.—It is the consensus of opinion among biological chemists that a starch determination must be preceded by an extraction of the material with dilute alcohol to remove the simpler carbohydrates. This holds not only in materials which are obviously relatively high in sugar and dextrins and low in starch, such as succulent tissues, but also in such materials as cereal grains, where the sugars are present in very small quantities (**14**). The concentration of alcohol and the details of procedure differ

[1] Separates of the various sections of the report of the Committee on Methods of Chemical Analysis of Plant Tissues can be purchased at nominal cost from the chairman of the committee, Dr. W. E. TOTTINGHAM, Agricultural Chemistry Building, University of Wisconsin, Madison, Wisconsin.

with the material involved,[2] but use of alcohol much more concentrated than 50 per cent. is liable to precipitate dextrins.

Gelatinization.—The residue from the alcohol extraction is freed from all but traces of alcohol, and to it is added sufficient water to make a thin suspension. The concentration of the suspension will depend on the starch content, and will be varied so as to allow for proper aliquoting for subsequent analysis. Boiling at ordinary pressure is used, and in general is to be recommended over autoclaving. If the material is ground rather fine, the latter treatment is unnecessary.

Saccharification.—Diastase in one form or another is very much to be preferred over acid for converting the starch to sugar, because of the more specific action of the enzyme. Takadiastase has been successfully used by several careful investigators (**6, 22**). Although HORTON (**12**) questions its reliability in some cases, it is generally conceded to be a trustworthy and convenient hydrolysant for analytical work. Salivary diastase was used by TOTTINGHAM and GERHARDT (**23**). The most commonly used source of diastase is barley malt. The principal precautions in its use are to obtain very active malt, to grind it fresh before extraction, to make careful blank determinations, and to test the solution for the absence of starch and dextrin.

When the analyst is satisfied that starch is practically the only polysaccharide in his material which is hydrolyzable by dilute hydrochloric acid, or when for other reasons he wishes to include some hemicelluloses in his hydrolysis, the diastase treatment may be replaced by heating the material at about 100° C. with about 2 per cent. hydrochloric acid, for from 1 to 3 hours. The strength of acid used has varied with different workers, but since the process is empirical the above average strength may be accepted as suitable in most cases. The time of heating employed has varied with the nature of the material; since this is also empirical it is either decided upon arbitrarily by the analyst, or, by means of trial hydrolyses, he determines the proper time for his particular material.

After saccharification of the starch it is customary to boil and filter the material, and then perform an acid hydrolysis on the filtrate. This is because all preparations of diastase contain some maltase; hence the hydrolysate contains a mixture of maltose and glucose, and a simple determination of reducing sugars will not suffice. DAVIS and DAISH (**6**) solved for the two sugars by means of a simultaneous equation. It is simpler, however, to remove interfering polysaccharides by filtration, or by alcoholic precipitation (**25**) and filtration, and then to hydrolyze the filtrate by boiling with approximately 2 per cent. hydrochloric acid for 2.5 hours, and determine the resultant sugar as glucose.

[2] See previous report of this committee, Plant Physiol. 1: 397–402. 1926.

Results should be reported in terms indicative of the method used; that is, as starch if diastase is used, otherwise as acid hydrolyzable material including starch.

Clarification.[3]—It has usually been considered necessary to clarify all sugar solutions before analysis by either polariscopic or chemical means. MORRIS and WELTON (**15**) question the necessity of clarifying all materials, as do WILLAMAN and DAVISON (**27**) in using the picrate method. The safest procedure is for the analyst to determine for his own material whether clarification is required. Tungstic acid and phosphotungstic acid (**18**) have been used to clarify sugar solutions, but neutral lead acetate is still the commonest reagent for this purpose. Deleading should be brought about with di-sodium phosphate, as it is least likely to cause the occlusion of sugars by the precipitate (**9**).

Determination of reducing sugars.—The analysis of the final hydrolysate can be carried out by any of the methods for reducing sugars given in a subsequent section of the report of this committee.

Hemicelluloses

It is regrettable that this ill-defined group of carbohydrates is still in a very unsatisfactory state as regards methods for its dissection and analysis. The group is roughly defined as including insoluble polysaccharides and related compounds which are hydrolyzed by boiling with dilute mineral acids; in other words, it contains pentosans, pectins, galactan and mannan. Some of these can be determined separately. The latter is greatly to be desired, and, indeed, its importance cannot be over-emphasized. The usual reason for embodying a number of these substances in the same analysis is that they represent reserve food materials in plant tissue. This, however, is largely an assumption, since we have only circumstantial evidence that this is the case. In any event, such group determinations give little indication of changes in proportion of constituents.

The group reagent for hydrolyzing the hemicelluloses differs with different investigators. Usually it is a dilute hydrochloric acid solution, such as 3 per cent. (**24**), 1 per cent. (**21**), and 0.5 per cent. (**22**). With some tissues, however, sulphuric acid seems to be preferable (**23**). The length of time of hydrolysis varies, but is usually from 1 to 4 hours. The conclusion seems to be warranted from the data at hand that each investigator should make a preliminary study of his material to ascertain what acid, what strength of acid, and how long a time of boiling are necessary to give the maximum amount of reducing sugars without appreciably affecting the true cellulose. Since the method is empirical, the following values are recommended either for direct use or as a basis for a series of trials: 2 per

[3] For details see report of this committee on determination of simple sugars.

cent. hydrochloric acid for 2.5 hours, at 100° C. These unknown factors and uncertainties emphasize the desirability of separating the individuals of this group, instead of employing a blanket method. After hydrolysis the solution is neutralized, clarified, and the reducing sugars determined by any method preferred.

Some progress is being made in the biological analysis of tissues, whereby specific substances are removed by enzymes or by micro-organisms. This appears to be a fruitful field for investigation, but no recommendations are as yet warranted.

Pentosans

There are two general methods for analyzing for the pentosans. One is the old "official" method of converting the pentosans to furfural and determining the latter in the distillate. The other is their hydrolysis to pentoses and the determination of the latter by copper reduction, after fermenting away the coincident hexoses. The first, or phloroglucinol method, is admittedly subject to many errors; but the only improvement on it, the method of PERVIER and GORTNER (**17**), is rather cumbersome to carry out. The fermentation method has been apparently successful in the hands of several investigators (**5, 21, 23**). This method is based upon the assumption that pentoses are not destroyed by yeast. Several strains of pure yeast have been found to attack the pentoses after fermentation of the hexoses, and hence the method is reliable only when the action of the yeast is stopped as soon as the fermentation of hexoses is complete.[4] Mrs. ABBOTT (**1**) has made an extensive study of the method and has found many pitfalls which must be avoided. The most important precautions are to use pure cultures of yeast, to adjust the medium to the proper acidity, and to test aliquots from time to time for the completion of the fermentation. Pure cultures can be secured from American Type Culture Collection, 637 South Wood Street, Chicago.

Galactan and mannan

These substances have not received much attention from the standpoint of analytical methods. Galactan is usually oxidized to mucic acid by means of nitric acid. Mannan is converted to mannose, and the latter quantitatively to its hydrazone. The reader is referred to the work of DORE (**7**) and of SCHORGER (**20**), respectively, for details of those methods.

Inulin

Inulin and the inulides are hydrolyzed to fructose, and the latter determined polarimetrically or by reduction (**13**).

[4] Private communication by C. O. APPLEMAN.

Pectins

The determination of the pectic substances is in an unsatisfactory condition, probably due to the fact that we have so little definite information about their composition. There was a time when one was limited to precipitating the pectin from solution by adding two volumes of alcohol, and weighing the precipitate. The latter contained protein, enzymes, and gums as well as pectin, hence the method was quite unsuitable. Then as more knowledge of the composition of the pectins was gained, other more specific methods were proposed.

VON FELLENBERG (**10**) proved that methyl alcohol is a constituent of all pectins; and as he believed it occurred in a constant ratio, he proposed to determine pectin by analyzing for methoxyl. It has been shown, however, that pectins from various sources differ in their methoxyl content, and hence this method is invalid. WICHMANN (**26**) proposed to saponify the pectin with alkali, precipitate the pectic acid with HCl, boil with the latter, and weigh the precipitated pectic acid. The objection to this method is the fact that furfural is always obtained by the action of HCl, and this represents a disintegration of the pectin molecule. CARRÉ and HAYNES (**3**) made exhaustive extractions of the finely ground tissue, usually as many as sixty extractions being required. The pectin thus dissolved was saponified, forming sodium pectate. The pectic acid was freed by acetic acid, and precipitated as the calcium salt. The latter was weighed as such. Besides being extremely laborious, this method is open to the criticism that the calcium pectate is probably not uniform in composition and that it occludes other substances. AHMANN and HOOKER (**2**) took advantage of the facts that soluble pectin has several carboxyl groups, either free or esterified, and that when pectin is boiled with excess of alkali a quantity of the latter will be absorbed which is proportional to the amount of pectin present. Their method is direct and simple, and gave results on pectin preparations comparable to those by the CARRÉ-HAYNES method. DORE (**8**), and NANJI, PATON and LING (**16**) boiled pectin with HCl so as to remove CO_2 from the carboxyl group of the galacturonic acid units of the pectin. The CO_2 was determined by weight or by titration. Although this was found to be an accurate method for galacturonic acid, it was not for pectin, probably because of a varying content of this substance in the pectin. Finally, CONRAD (**4**) has developed a way of extracting the insoluble pectic compounds of a tissue with ammonium citrate and with very dilute HCl, and determining the resultant pectic acid by the method of CARRÉ and HAYNES.

It is impossible for the present committee to make a definite recommendation. The AHMANN-HOOKER method appeals because of its simplicity. It is probably more important at present to investigate the pectin molecule and to devise methods than it is to try to analyze tissues quantitatively for pectins.

This report was organized by J. J. WILLAMAN for the Committee.

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W. E. TOTTINGHAM (chairman),
J. J. WILLAMAN.

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BRIEF PAPERS

APPARATUS FOR CONTINUOUS DIALYSIS AT LOW TEMPERATURE

(WITH ONE FIGURE)

The authors have had occasion to carry out on a considerable scale the dialysis of plant tissue fluids, and have found the apparatus here described very convenient and effective. It can be used at any temperature, but in our work has been used in conjunction with a cold chamber to check enzyme action in the juice.

The supply of distilled water is provided by an ordinary still kept running during dialysis. The water enters at *W* (fig. 1), dropping through a layer of toluene *T* floated on the surface of the water in *R*, a carboy. *R* and *R'* act as reservoirs. The dialyzing system is enclosed in *F*, a "frigidaire" box in which the cold temperature is automatically held within fairly close limits. The water is cooled by passing through *C*, which may consist of a coil of block tin tubing, or a number of bottles in series. The bottle *B* is provided with a siphon adjustable for height at *A*. This siphon is of relatively small bore and requires 3 or 4 minutes to empty the contents of *B* into the diffusion vessel *V*. The latter has a large-bore siphon, which empties the contents of *V* into the drain *D* in less than one minute. The inner end of the large siphon drops into a small sump in the bottom of *V*, thus insuring the complete emptying of this vessel.

The diffusion vessel *V* is filled with water to the bend in its siphon. When *B* begins to discharge into *V*, the large siphon of the latter vessel is automatically brought into operation. The quick draining of *V* breaks the action of its siphon a few moments later, allowing the vessel to be refilled at once by the remaining discharge from *B*. The frequency of water changes in the diffusion vessel is regulated by the stopcock *S*, and also by drawing out to a small bore the end of the supply tube entering *B*. After the system has been adjusted, we have found that it will continue without further attention, changing the water once every hour for several days.

The size of the different containers may be varied to suit conditions. In our work the diffusion vessel accommodates twelve dialyzing tubes, each of 100 cc. capacity. Any change in the number or volume of the tubes used would of course necessitate a change in the adjustment of the upper siphon at *A*. In practice we have found it more convenient to keep the number of tubes constant, simply filling with water those not required in any particular experiment.—R. NEWTON and W. M. MARTIN, *University of Alberta*

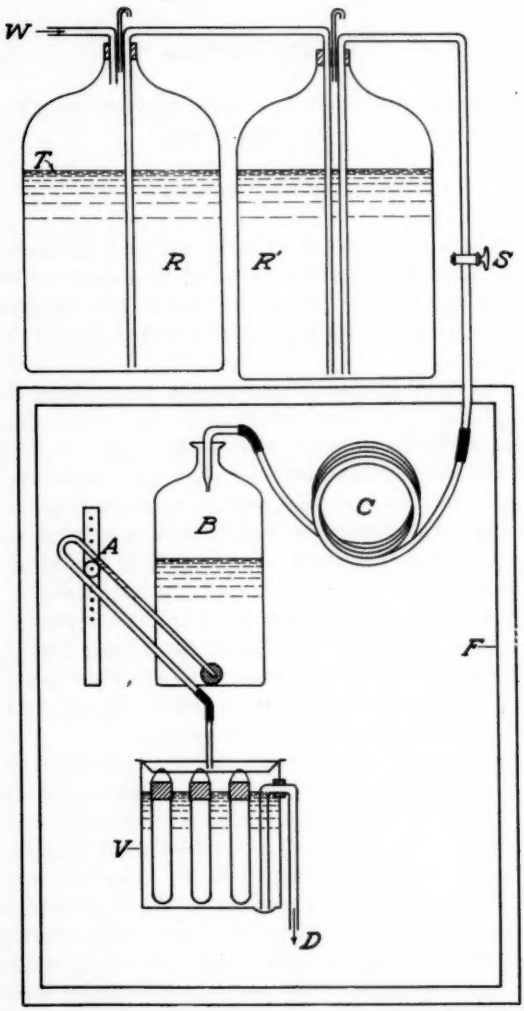

FIG. 1. Apparatus for continuous dialysis at low temperature.

A LABORATORY METHOD OF PREPARING STARCH FROM MAIZE SEED[1]

Preparations of maize starch of a high degree of purity have been obtained from the seed by adapting the essential processes involved in the commercial manufacture of this product to laboratory conditions. The method may be extended to other grains by modification of certain steps in accordance with the nature of the fatty and proteinaceous substances present.

Clean maize seed placed in battery jars is steeped in a solution of sulphurous acid of a concentration of 1° B. (1.007 sp. gr.) for 24 hours at a temperature of 40° C. At higher temperatures, not to exceed 60° C., the time may be shortened. During the period of steeping, the strength of the solution is kept at approximately 1° B. by addition of further sulphurous acid from a stock solution. The sulphurous acid affords antiseptic conditions, has a pronounced bleaching effect, and so softens the grain that the starch is readily separated from the materials in which it is embedded.

The softened grain is then washed with water and ground in a meat grinder fitted with medium plates. Binding in the machine may be prevented by the addition of a little water from time to time. By kneading the wet pulp in a double layer of cheesecloth the starch fraction is worked through, leaving most of the embryo and pericarp tissue behind. A considerable portion of the remaining non-starch particles is removed by washing with water on a metal sieve having 100–120 meshes per inch. The crude starch is allowed to settle and the supernatant liquid is siphoned off.

Purification is effected by treatment with alkali. Enough water is added to the crude starch to make a flowing paste. This paste is poured slowly, with constant stirring, into about 5 volumes of a solution of NaOH not exceeding 0.45 per cent. in strength. A concentration of 0.70 per cent. NaOH gelatinizes starch very quickly and makes the separation of impurities very difficult or impossible. The mixture should be well stirred for half an hour, and further agitated at intervals over a period of 3 or 4 hours. The starch is allowed to settle, and the supernatant liquid containing the major portion of the impurities soluble in dilute alkali is siphoned off. The solid residue is washed with water and again allowed to settle.

The partially purified starch is taken up with distilled water and stirred thoroughly. If allowed to settle for a period of 10 to 15 minutes the coagu-

[1] Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 69. Published with the approval of the Director of the Station.

lated alkali-insoluble proteins fall to the bottom and the supernatant liquid, containing the starch in suspension, is siphoned off. This process is repeated three times. Alkali-soluble impurities still present, and the NaOH, remaining, may be removed by again suspending the starch in water and allowing it to settle 3 or 4 times over a period of two days. The last wash water should be clear and give a P_H of 6 to 7.

Finally the starch is placed over filter paper in a Büchner funnel and treated with 95 per cent. alcohol followed by successive portions of absolute alcohol to dehydrate. Fatty substances still present are removed by washing the water-free starch with ether. The starch is dried in crystallizing dishes at approximately 40° C.

Analysis of a sample of starch prepared by this method from common maize showed 0.035 per cent. nitrogen. A lot of waxy maize starch, which stains reddish-brown with iodine, prepared side by side with the above sample contained 0.013 per cent. nitrogen.—R. A. Brink and F. A. Abegg, *University of Wisconsin, Madison, Wisconsin.*

THE ACTION OF ETHYLENE IN ACCELERATING THE BLANCHING OF CELERY

Preliminary experiments to date have been concerned with the following points:

1. The effects of various concentrations of ethylene on the rate of blanching, on the crispness and flavor, and on the storing quality of the celery.

2. The effect of the treatments on the acidity of the celery juice, as an indicator of physiological changes in the plants.

3. The action of ethylene on the rate of respiration of the celery.

4. The effect of removal of carbon dioxide, a product of respiration, on the blanching process, and also the effect of excess amounts of carbon dioxide, as an index of the rôle of by-products of respiration.

Concentrations varying from one part of ethylene in 500 of air, to one part in 50,000 of air, have been used. The most rapid and complete blanching took place where the lower concentrations were used. Very little difference was apparent between 1:25,000 and 1:50,000; these concentrations, however, were much superior to the stronger ones (1:2500 to 1:500), which showed little more blanching than the untreated check. The larger amounts of ethylene, particularly the concentration of 1:1,000, produced an injury characterized by splitting of the stalks on the inner surface, and pronounced pithiness of the stalks thus split.

The acidity of the celery juice was apparently not changed by the ethylene treatment. Differences in flavor were evidently not caused by the accumulation within the plants of by-products of respiration.

The rate of respiration was more than doubled by exposure to ethylene, as compared to the rate for untreated celery. The most rapid respiration, as indicated by the amount of carbon dioxide given off, took place in the sample exposed to one part of ethylene in 50,000 of air. In other words, the most rapid respiration was accompanied by the best blanching. Samples exposed to this concentration of ethylene were the most crisp and tender, but were the most subject to rot.

The removal of carbon dioxide by the use of calcium oxide and potassium hydroxide increased the rate of blanching and also the amount of rot. In the presence of very high concentrations of carbon dioxide, ethylene at the rate of 1:10,000 failed to produce any blanching at all.

These facts indicate that ethylene accelerates the blanching process by stimulating the activity of enzymes normally concerned with the breaking down of various compounds in the plant. With this in view, further studies are being initiated on the effect of ethylene on the enzymes of the celery plant, particularly oxidase, peroxidase, catalase, protease, cellulase, and pectase.—W. B. Mack, *Division of Vegetable Gardening, Department of Horticulture, State College, Pa.*

NOTES

The Philadelphia Meeting.—The third annual meeting of the American Society of Plant Physiologists was held at Philadelphia, December 28–31, 1926. The various sessions were held in the Veterinary Laboratories of the University of Pennsylvania, and in various other buildings in connection with joint meetings with Ecologists, Horticulturists, and the Physiological Section of the Botanical Society of America. The meetings were well attended, even though the meeting room was located inconveniently for those who desired to migrate from one meeting to another. The meeting Tuesday morning, December 28, was one of the finest ever held by any biological group, and set a standard that will be difficult to excel. The program committee deserves high praise for its work in arranging the invitation papers and others in groups somewhat related as to subject matter. The only regrettable feature was the fact that two groups of plant physiologists were meeting simultaneously, so that no one could hear all the papers. If all such meetings could be made joint meetings, held in a single room, so that anyone could hear all the papers if he so desired, it would be a good step forward.

Summer Meeting.—For two years the Corn Belt Section of the American Society of Agronomy and the Physiologists have held joint meetings in July. It had been proposed to hold a meeting at Purdue University this summer, but in view of the fact that the Agronomists will probably hold a meeting there within a year or two, the executive committee decided that it would be better to hold no summer meeting in 1927. This leaves us free to concentrate upon the next annual meeting, to be held at Nashville, Tennessee, in December, 1927. Every member should plan to attend the Nashville meeting.

Life Membership Award.—The first award of the CHARLES REID BARNES Life Membership was made at the banquet of the American Society of Plant Physiologists on the evening of Wednesday, December 29, 1926. The Society, at the suggestion of the committee charged with making the selection, has conferred the honor of life membership in the Society, *honoris causa*, upon Dr. BURTON EDWARD LIVINGSTON, Professor of Plant Physiology at the Johns Hopkins University since 1909, and Permanent Secretary of the American Association for the Advancement of Science since 1920. A full account of the award has been published in *Science*. Each year a new link in this living memorial to Dr. CHARLES REID BARNES will be added, and the banquet should prove a happy and appropriate time for making the announcement of the award.

Errata.—An occasional error creeps into every publication. The ideal of the Editorial Committee of this journal is to produce a publication as nearly free from mistakes of any kind as is humanly possible. We call attention to the small list of *errata* listed in connection with the table of contents of Volume I. Those who possess the journal should make the corrections at the place where they occur. We invite all of our readers and contributors to call to the attention of the editors any error found in PLANT PHYSIOLOGY, in order that proper corrections may be made.

Condition of the Society.—The growth of the American Society of Plant Physiologists has been truly remarkable during the past year, and the new year starts under the most favorable auspices. The membership numbers nearly 250, and the library subscriptions exceed 100 at the beginning of the second year of publication. The income of the Society during 1926 was more than sufficient for current needs, and we have a small surplus of Volume I, which will bring some hundreds of dollars into the treasury when completely sold out. Few scientific organizations have ever had such a promising start, in connection with the publication of a high class journal.

Back Numbers.—Institutions, libraries, and individuals who may desire to own a complete file of PLANT PHYSIOLOGY should take steps at once to acquire the first volume. The conservative policy of the Executive Committee and the Editorial Committee led to the publication of only a small number of copies of Volume I, a strictly limited edition. The surplus left is not nearly large enough to give each American plant physiologist the opportunity to own one. The large demand for it was not anticipated, and as a result there is imminent danger of exhaustion of the first volume. Those who are interested in Plant Physiology should not wait to be invited to join the Society, but should write to the Secretary, Dr. S. V. EATON, Department of Botany, The University of Chicago, and make application for membership. Ten dollars will procure volume I while it lasts, and give the member all of the 1927 issues.

Fifth National Colloid Symposium.—The fifth National Colloid Symposium will be held at the University of Michigan, Ann Arbor, June 22–24, 1927. It has been customary to invite some distinguished investigator as the guest of honor. This year Dr. H. R. KRUYT, of the University of Utrecht, has been invited to lead the discussions. These meetings have been very stimulating in the past, and biologists will find much of value in the discussions. Anyone interested in colloidal phenomena is welcome to attend the meetings. There are no fees of any kind, and the fellowship is un-

usually cordial among the attendants of these symposia. Dr. KRUYT will remain at the University of Michigan for the summer session and will offer an opportunity for special courses in colloid chemistry.

Hydrogen Ion Concentration.—The second German edition of L. MICHAELIS's Wasserstoffionenkonzentration has been translated into English by Dr. W. A. PERLZWEIG, of Johns Hopkins University, and thus a most excellent book becomes generally available to the students in this country. Already familiar to many of us in the German, the book needs no detailed description. The first part deals with the chemical equilibrium of the ions, and the second part with the ions, particularly the hydrogen ions, as sources of electric potential differences. Each part consists of five chapters. The book considers the theoretical problems of equilibrium and potential difference in a very helpful way, and supplements in an excellent manner the manual by CLARK. It is published by Williams and Wilkins, Baltimore, Md., in handsome binding. Price, $5.00.

Manual of Plant Diseases.—The best text yet produced for the student of phytopathology has been written by Dr. F. D. HEALD, of the Washington State College. The book is also of considerable interest to plant physiologists because it contains a number of chapters on the so-called physiological diseases of plants. Section II is entitled "Non-parasitic Diseases," and contains chapters on diseases due to deficiencies of food materials in the soil; diseases due to excesses of soluble salts in the soil; diseases due to unfavorable water relations; diseases due to unfavorable air relations; diseases due to high temperatures; diseases due to low temperatures; diseases due to unfavorable light reactions; diseases due to manufacturing or industrial processes; and disease due to control practices. There is also a section on virus diseases. From this classification one can see that there is no such thing as an undiseased plant.

Physiologists will probably take the point of view that these matters belong, also, in texts on plant physiology. Certainly the material ought to be a part of the physiological work in every laboratory in the country. These diseases after all are only the "normal" response of the plant to the given environment, and progress in the elucidation of these physiological responses will be more rapid if well trained physiologists take an interest in the investigation and presentation of the facts regarding these causes of physiological breakdown. Physiologists have been too slow to take advantage of the practical and economic aspects of their work.

The book, which is excellently done, should be a possession of all who desire to keep abreast of the advances in the field of phytopathology. The price is $7.00, and the publishers, McGraw-Hill Co.

Palladin's Plant Physiology.—The LIVINGSTON translation of this work has appeared in the third English edition. It is essentially the same as the second edition, but has had the editorial notes revised to include some more recent references. The list of cited literature at the beginning of the volume has been nearly doubled in size, and the book will no doubt continue to be useful to students of plant physiology. P. Blakiston's Son and Co. are the publishers, and the price is $4.00.

Surface Chemistry.—Surface phenomena play a very large rôle in biological processes, and this "Introduction to Surface Chemistry," by Professor ERIC K. RIDEAL, Humphrey Owen Jones Lecturer in Physical Chemistry at Cambridge University, should be a very useful volume to the physiologist. The preface is by Dr. F. G. DONNAN, of University College, London, who emphasizes the importance of the "two dimensional" molecular world, the existence of which has been revealed by the pioneer work of Lord RAYLEIGH, MARCELIN, HARDY, LANGMUIR, ADAM, and RIDEAL. The survey of this rapidly developing field is admirably executed, and the book should be in the hands of all whose studies involve a knowledge of surface chemistry.

The first two chapters deal with surface tension of liquids, and solutions, while the third chapter discusses the surface films of insoluble materials. The succeeding three chapters take up the interface phenomena in liquid-liquid, gas-solid, and liquid-solid interfaces. The seventh chapter deals with differences of potential at interfaces, and chapter eight considers the problems of stability in suspensions and emulsions. The final chapter is devoted to gels and hydrated colloids, such as silica gel, gelatine, proteins, soaps, and colloidal dyes.

The book is published by the Cambridge University Press, and may be ordered from the Macmillan Co., New York. Price, $5.50.